QA/QC for Wastewater Laboratory Testing

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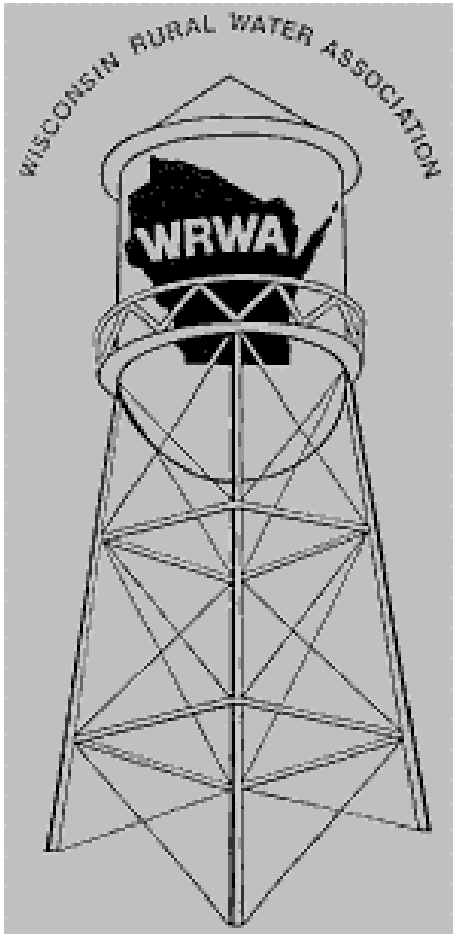

WSLH WISCONSIN STATE LABORATORY OF HYGIENE

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OVERVIEW

- Why QC?
- What’s in it for me?
- QC vs. QA ...what’s the difference?
- General Components of a QA/QC Program
- Examples of QC
- Setting up an Effective QA Plan

Quality Contorl

Why QA/QC?

Fundamental concept applies far beyond laboratory

Would you .... buy used car from a car rental company?

.... drive cross-country before checking the oil, etc.?

Common sense

"Checks and balances" is a universal concept. (aircraft)

"To err" is human (double key entry)

Prove data valid mostly through documentation

"He who has the best documentation wins."

Important to data user **<u>and</u>** the laboratory

User wants to make correct decisions....and sleep at night!

Laboratory wants to produce a good product....and pass audits!

Yeah....but what's in it for me?

What's in it for me?

⇒ Millions spent on plant upgrades based on lab data
(Consider the plight of plant designed and built using inaccurate flow measurement data that is later *determined to be undersized)*

⇒ Better data can result in reduced NR 101 fees

⇒ Needed to maintain lab certification/registration

⇒ Quality data required to show plant functioning properly

⇒ Policy and guidelines promote uniformity

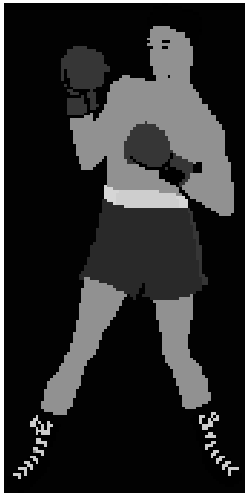
⇒ Decreased learning curve for new employees

⇒ Fewer repeated analyses

⇒ And.....?

OK, so QC and QA are important...but aren't they the same thing?

QC v. QA



QC

Specific
technical, operational
measures or activities
to ensure lab data quality.

QA

General
management function
to ensure data quality
relies on:

- documentation and establishment of QC protocols,
- **evaluation** and summarization of their outcomes

QC vs. QA

	QC	QA
100 mg weight = 83 mg	✓	✗
spike control limits 90-110%	✓	✓
barometer reads 28.4 inches	✓	✗
no spikes for ammonia, TP	✗	✗
TP spike limits -275 to 3047%	✓	✗
TSS oven temp. kept 180 $\pm$ 1^{o}C	--	✗
Filling out benchsheets in advance *dates and names in advance of collection*	✓	✗

With clear distinction....you can build a QA/QC program

Components of a good QA Program

The foundation

- Good facilities and equipment
- Training of personnel
- Operation plan (assigned responsibilities)
- Methods documented and followed

The structure

- Rigorous QC procedures
- Precision
- Accuracy
- Documentation to ensure traceability

A strong structure requires the use of the right materials.....

Type and Uses of QC samples

Blanks

- Laboratory reagent water.
- Used to verify the absence of contamination in the lab.
- Particularly important in phosphorus and ammonia testing.

Known Standards

- Used to verify calibration curve accuracy, or
- absence of bias in laboratory procedure (vs. matrix-effects)
- best if these are prepared from a different standard than is used for calibration standards.

Replicates

Used to measure the ability to reproduce your results.
You got it right once, but can you do it again?

Type and Uses of QC samples

Spikes

Used to evaluate bias

(i.e.., the recovery of the analyte from the specific sample matrix).

If you only get 25% spike recovery,

.....and your sample concentration is close to a permit limit

....isn't it likely the permit limit has actually been exceeded?

Reference Samples

- Annual requirement
- "Show me you can do this test right"

Blind Standards

Same as reference samples, but more timely.

But the materials must be used correctly to serve their purpose: precision and accuracy

Precision & Accuracy

Accuracy (Bias)

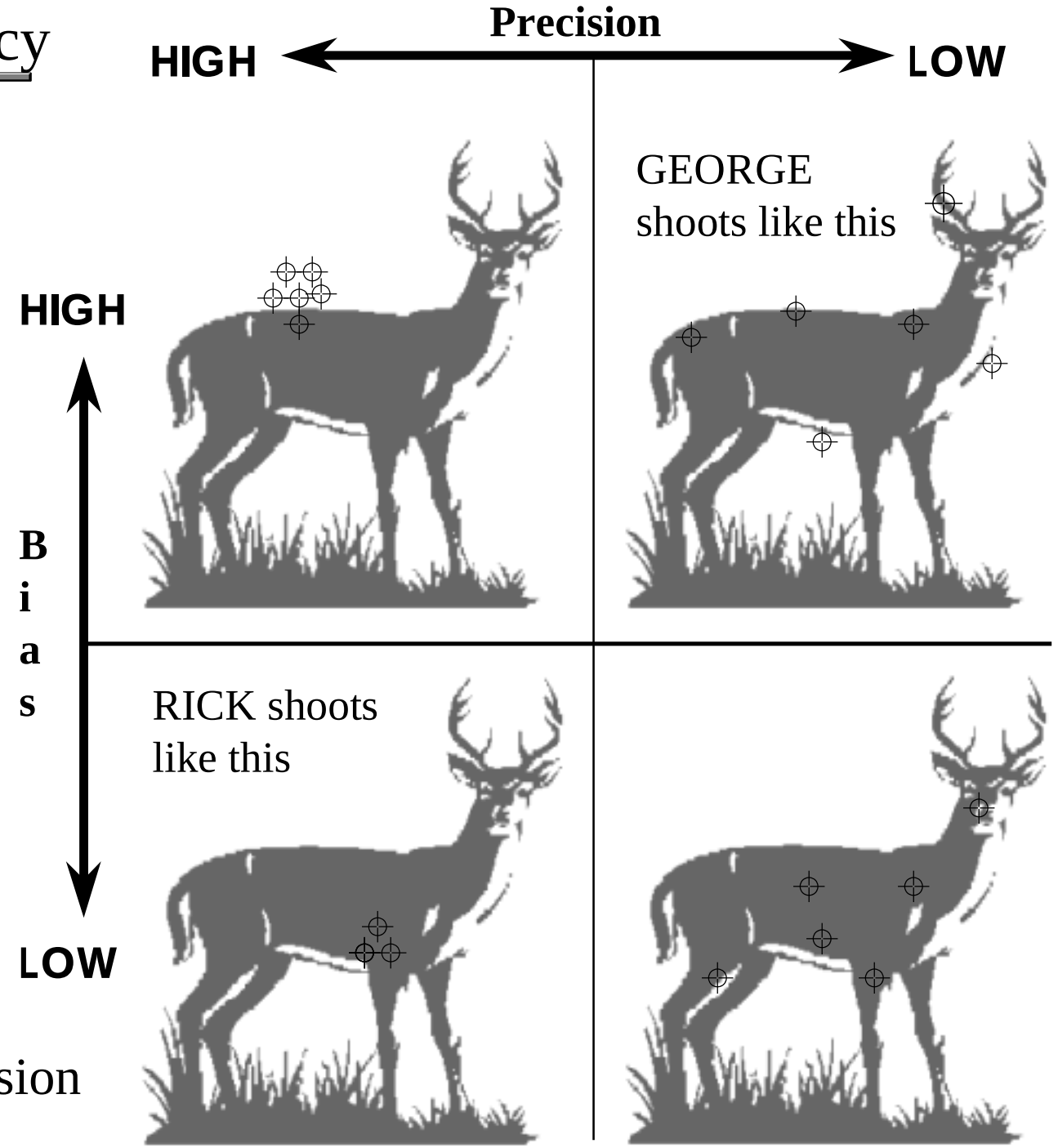
How close you can get to the true value.

You want LOW bias
(bias is not a good thing)

Precision

Reproducibility of the method. The ability to get the right answer - again

You want HIGH Precision

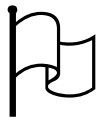


Pitfalls of Poor P&A

☹ Report results that violate discharge limit... (when they actually didn't!).

$ Increased NR 101 fee$.

 Periodic, unexplainable limit violations.

 Bring overall ability to operate plant into question.

Setting up an effective QA Plan

- ✓ **Standard Operating Procedures (SOPs) should be available for anything not self-explanatory**

  Ex. How do you clean the phosphorus glassware?

  Essentially, if someone unconnected to the lab were to perform this task, what guidance would they need to do it?

- ✓ **You can simply reference SOPs, rather than including them in your QA Plan**

- ✓ If you don't do it, DON'T include it in the QAP.

Many QA Manuals are merely loaded with marketing "fluff" that doesn't say much

QA Plan "DON'Ts"

DON'T allow your QA Manual to read like ....

The "baffle them with really serious, business-type words" approach

Quality Assurance is a systematic design plan incorporating a number of related laboratory aspects.

We know what QA is, but it's too complicated to explain here.

.....

The "We do some really high-tech stuff here" approach

Accurate and precise analytical data can only be realized by systems that are capable of comparing the response of a real world sample to the response of a known standard.

First you have to calibrate.

Setting up an effective QA Plan

- Tables are better than lots of text!
  - ✓the old “a picture is worth 1000 words” concept
  - ✓Tables FORCE you to be brief

3 rules for building a QA Plan by tables

What am I looking at? (parameter)

What am I looking at it for (criteria)

What if it doesn’t meet specifications? (Corrective Action)

Sound easy enough? Let’s see some real-life examples.....

Setting up an effective QA Plan

Evaluating?	**Criteria**	**Corrective Action**
Method Blank	Below LOD	1) Identify source 2) Correct & Reanalyze 3) or Qualify data
Known Standard	Within 90-110%	1) Check prep. data 2) Re-make & re-analyze 3) Make new curve
Matrix Spike	Within Control Limits (80-120%)	1) Re-make & re-analyze 2) Analyze known std. 3) Qualify data

QA PLANS - The Bottom Line

brief NOT volumes

realistic NOT marketing "fluff"

Guidance NOT Philosophy

decision trees NOT generic options

reference NOT paperweight

tables NOT text

Quality ~~Contorl~~ Control

Calibration

CALIBRATION - Discussion points

- Initial vs. continuing calibration
- How many standards to use?
- To include...or not to include (a blank)?
- Processing the data
  - » internal calibrations
  - » graph paper
  - » linear regressions
  - » software
- Evaluating a calibration
  - » visual
  - » statistical
  - » analytical

CALIBRATION - Initial Considerations

Frequency

- For best results, should be run daily.
- Alternatively, a “full” calibration can be analyzed initially and verified (with one or more standards) each day of analysis.

Use an appropriate number of standards

Calibrations must be constructed using at least 3 standards and a blank.

Know when to include a zero

- A good rule of thumb: if you can adjust your instrument to read zero in the presence of a blank, then include a zero point in your calibration curve.
- Including a zero is generally appropriate for colorimetric procedures

CALIBRATION - Initial Considerations

Define your calibration range properly

- Range should be appropriate for the samples being analyzed (i.e. don't calibrate from 1- 5 mg/L when all samples are between 0.05 - 0.5 mg/L).
- Better results are obtained when sample response is close to response of standards used to establish the calibration curve.
- Optimal results ==> when sample results fall near the mid-point.
- Standards should also be evenly spaced.

  1, 2, and 500 are NOT good levels for a calibration

- Whenever possible….bracket samples with calibration standards.
- Low standard not more than 2 to 5 times the LOD (best is = LOQ).

CALIBRATION - Processing the data

Pre-programmed Calibrations

⇒ Use of pre-programmed calibrations is unacceptable

⇒ Laboratory must generate its own standard curve.

NOTE: A manufacturer's claims that their method is approved or acceptable does not mean that the approval extends to pre-programmed calibrations.

Hand-drawn Calibration Curves

⇒ Plot concentration on the x-axis and absorbance on the y-axis.

⇒ A straight line which best fits the data points is then drawn.

⇒ The "best fit" line used to convert absorbance into concentration.

⇒ Makes traceability virtually impossible

- Significant variability in how the scale of the graph is constructed
- Significant variability in how any individual draws the "best fit" line

CALIBRATION - Processing the data

Scientific Calculators & Software

⇒ Using a standard procedure can eliminate sources of variability.

⇒ Linear regression = one of the most widely recognized means.

⇒ Linear regression equations can be generated.....
.....with an inexpensive scientific calculator
.....or most spreadsheet programs.

Calibration exercises

1. Graph paper

Make a calibration graph from:

Calibration Data	
mg/L P	Abs.
0	0
0.1	0.051
0.5	0.25
2	0.72
5	1.24

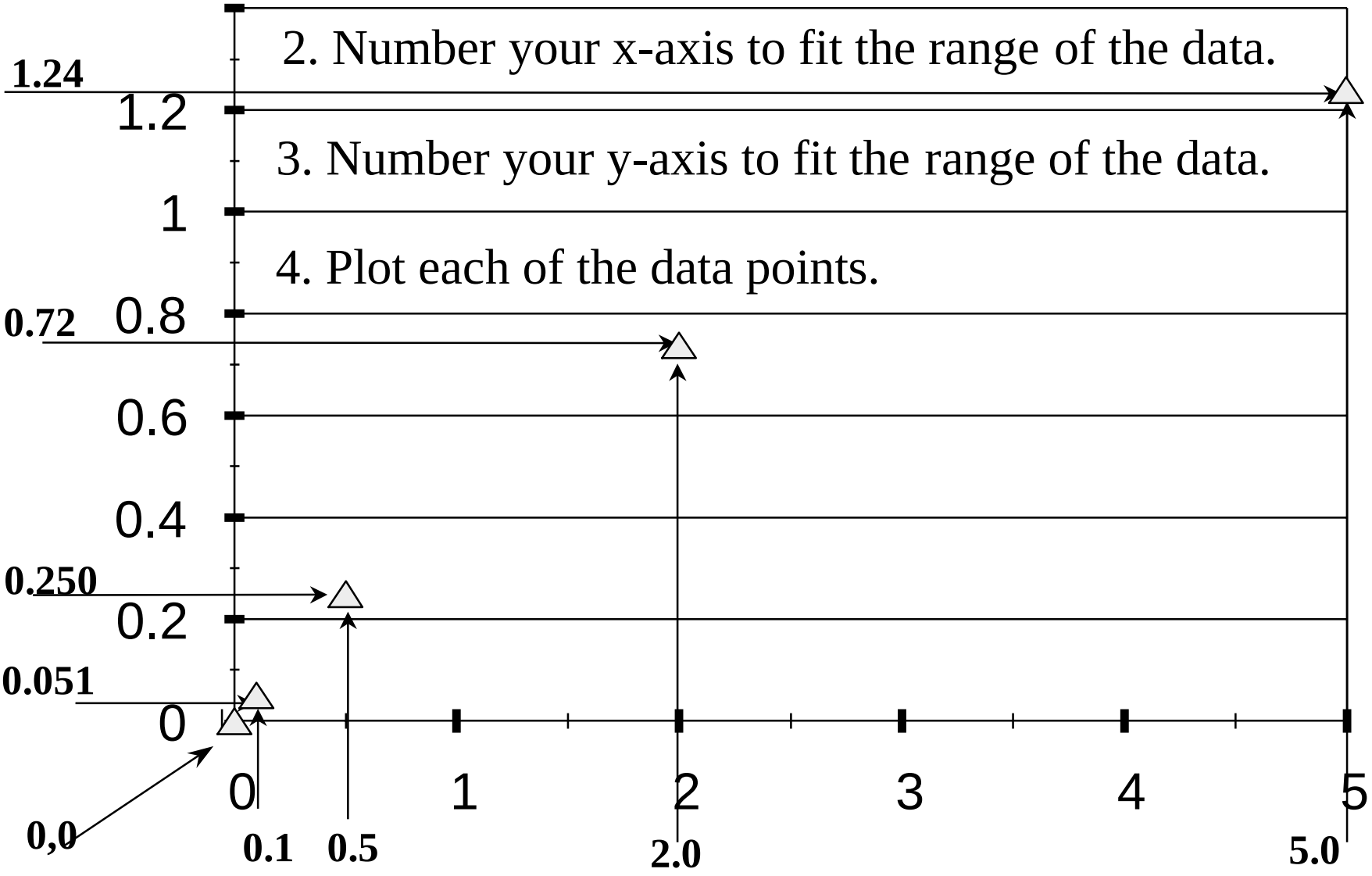
....then

Find Concentrations for these Absorbances

0.118
0.531
0.770
0.853
1.092

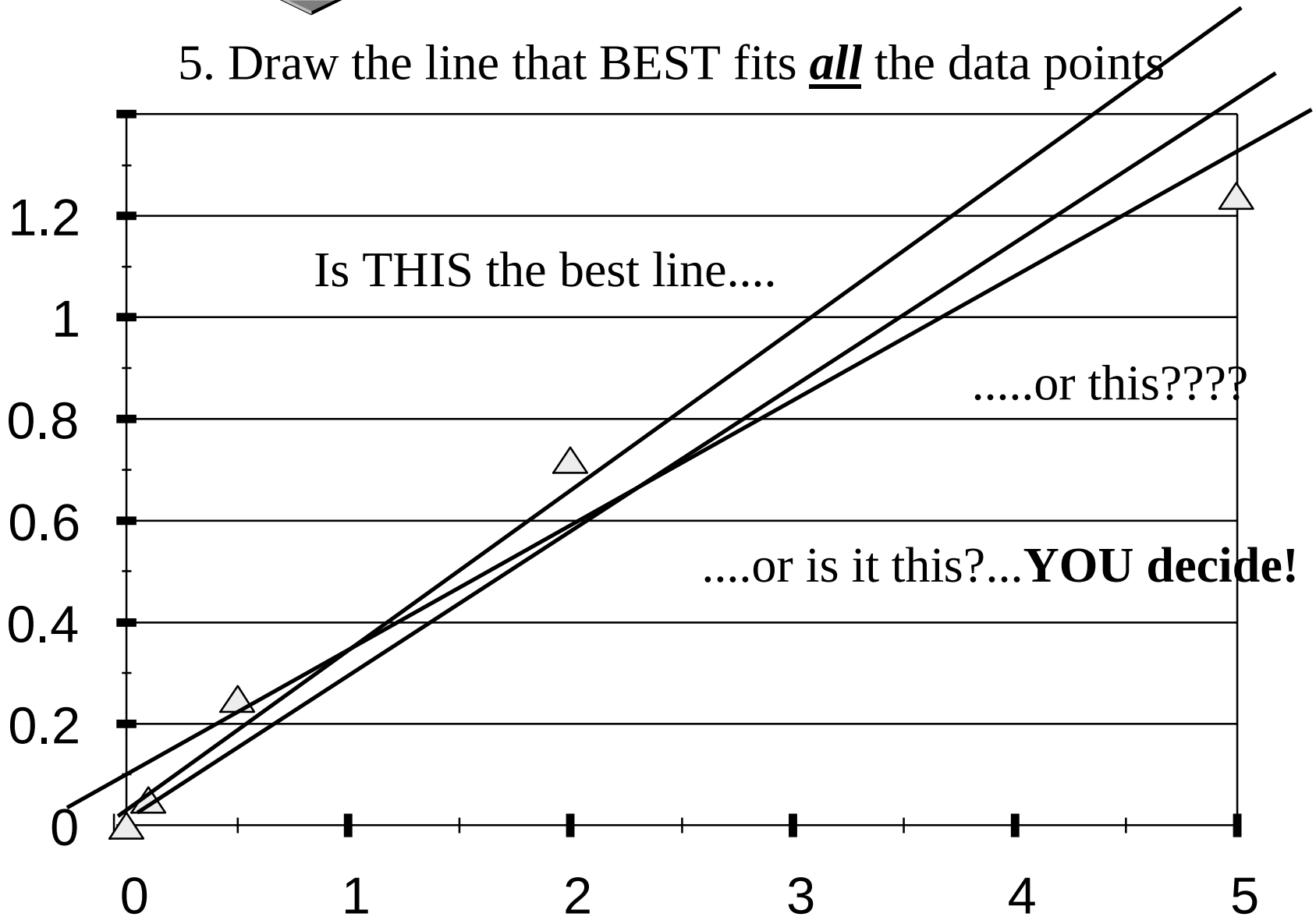
CALIBRATION - Processing the data by graph

1. Draw the X and Y axis (graph frame)

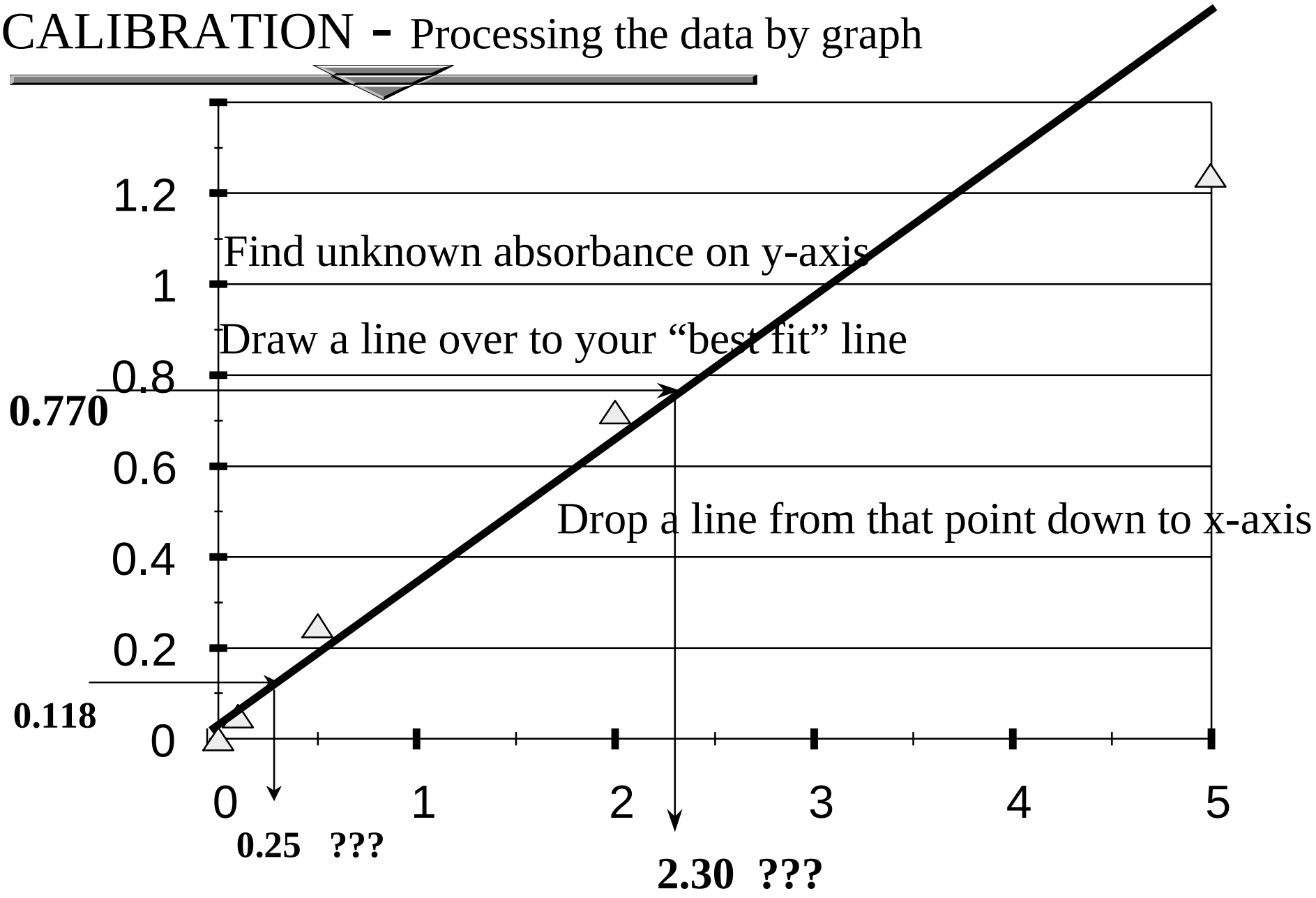


CALIBRATION - Processing the data by graph

5. Draw the line that BEST fits ***all*** the data points



CALIBRATION - Processing the data by graph



Find unknown absorbance on y-axis

Draw a line over to your “best fit” line

Drop a line from that point down to x-axis

...and read concentration off the x-axis.

CALIBRATION - Processing the data by graph

Observations

Some prepared graph landscape  vs. portrait 

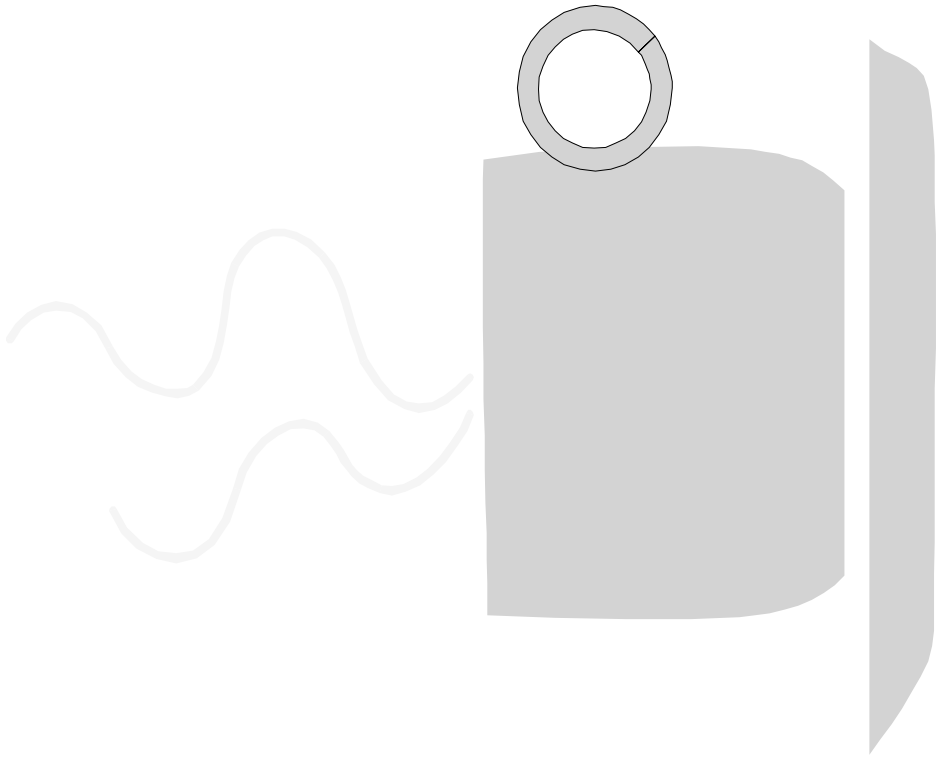
Some used the whole page, others just part of the page

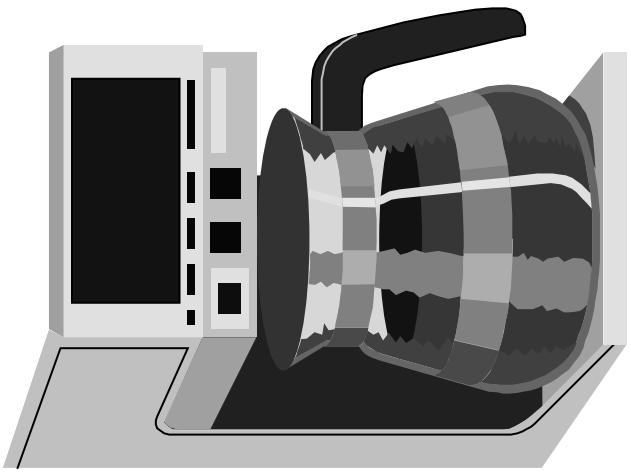
Quite a bit of spread in the data....range of 1 ppm or more

Some read to nearest 0.01 ppm; others to nearest 0.1

Food for thought....

What if your permit limit is right about 2 ppm?
Should the line pass through the origin (0,0)?





Calibration exercises

2. Linear Regression

Calculate a regression line from:

Calibration Data	
mg/L P	Abs.
0	0
0.1	0.051
0.5	0.25
2	0.72
5	1.24

....then

Find Concentrations for these Absorbances

0.118
0.531
0.770
0.853
1.092

CALIBRATION - Using the Sharp EL-520 Calculator

Basics

- MODE button: **0** = basic calculator, **1** = mean & SD, **2**= regression
- 2nd F button (yellow, upper left of calculator).
- **2nd F** and **DEL** button clears memory...do this several times to be sure!
- STO button: adds the "X" component of an XY data pair
- M+ button: adds single datum or 2nd of a data pair to memory.
- RCL button: think "Re-call"; retrieves information.
- *****You may want to use a pen or pencil to push buttons!******

- Calculator instruction sheets include instructions on how to turn the calculator off.
- Don't need to do this now...but you should be know how to do it if you need to.
- Calculators are battery-powered with solar-powered back-up.
- Will automatically shut-off after about 10 minutes if not being used.

CALIBRATION - Entering Regression Data into Calculator

Calibration Data

mg/L P	Abs.
0	0
0.1	0.051
0.5	0.25
2	0.72
5	1.24

Calculator steps

Set Mode to "2": MODE [redacted]

Clear the registers: 2nd F DEL

Enter 1st data pair: [redacted] **STO** [redacted] **M+**

Enter 2nd data pair: [redacted] **STO** [redacted] **M+**

Enter 3rd data pair: [redacted] **STO** [redacted] **M+**

Enter 4th data pair: [redacted] **STO** [redacted] **M+**

Enter 5th data pair: [redacted] **STO** [redacted] **M+**

CALIBRATION - Regression using the Calculator

Obtain the calibration evaluation information

- ✓ Get the Correlation coefficient..: RCL ÷ r = 0.982442272
- ✓ Get the Slope.....: RCL) b= 0.244713124
- ✓ Get the Intercept: .....: RCL (a = 0.080236051

Converting a sample absorbance to concentration

Absorbance = 0.118: [redacted] 2nd F (**0.154**

Absorbance = 0.531: [redacted] 2nd F (**1.842**

Absorbance = 0.770: [redacted] 2nd F (**2.819**

Absorbance = 0.853: [redacted] 2nd F (**3.158**

Absorbance = 1.092: [redacted] 2nd F (**4.134**

CALIBRATION - Regression using the Calculator

Value of the Correlation coefficient (r)

- Looking for $r \geq 0.995$
- Tells you how closely points fit the regression (best fit) line

Value of the Slope (b)

- With electrodes, helps tell condition of the electrode (-54 to -60)
- Can keep records to show when the analysis is changing

Value of the Intercept (a)

- Represents the concentration associated with NO (0) response
- Thus gives an approximation of detection limit

if your intercept exceeds your LOD, there may be contamination

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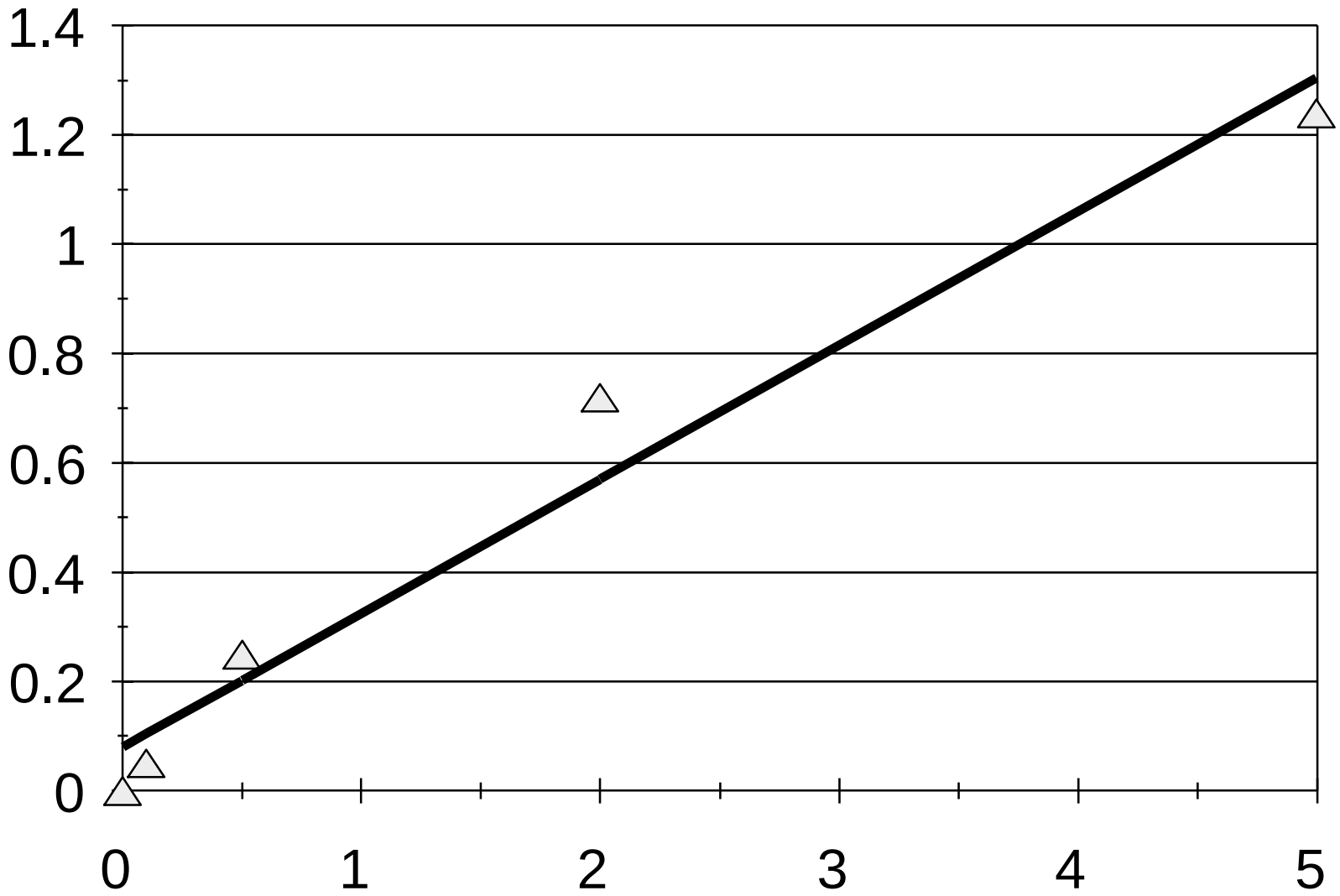
The ONLY downside to using a calculator vs. a spreadsheet program is that you do NOT get the visual evaluation power afforded by charting the data and regression line.



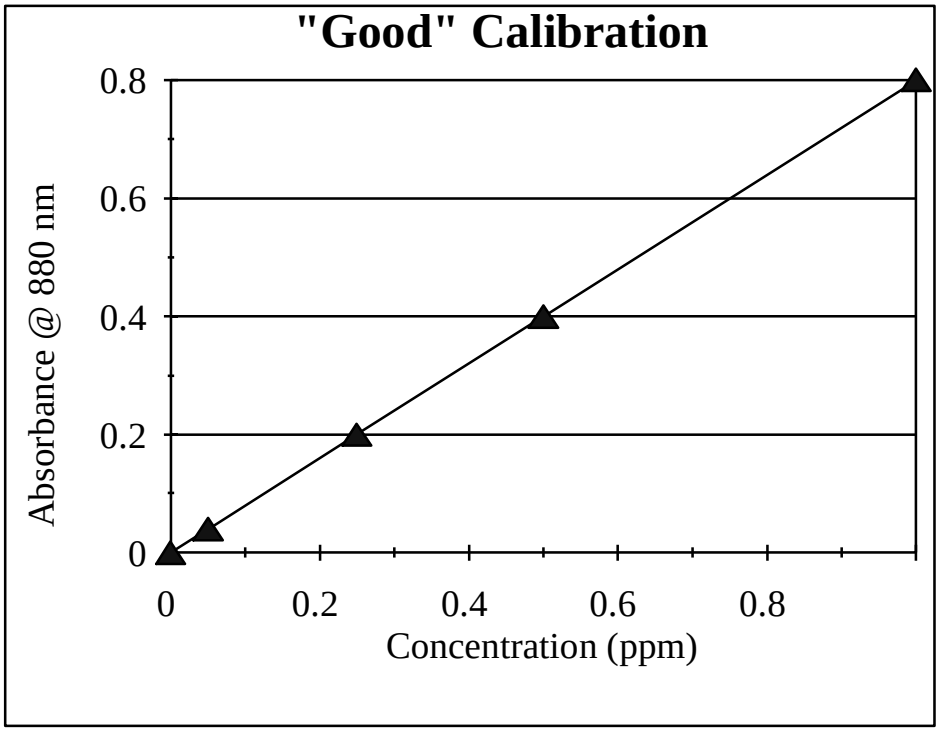
Abs.	**0.118**	**0.531**	**0.77**	**0.853**	**1.092**
Regression	0.15	1.84	2.82	3.16	4.13

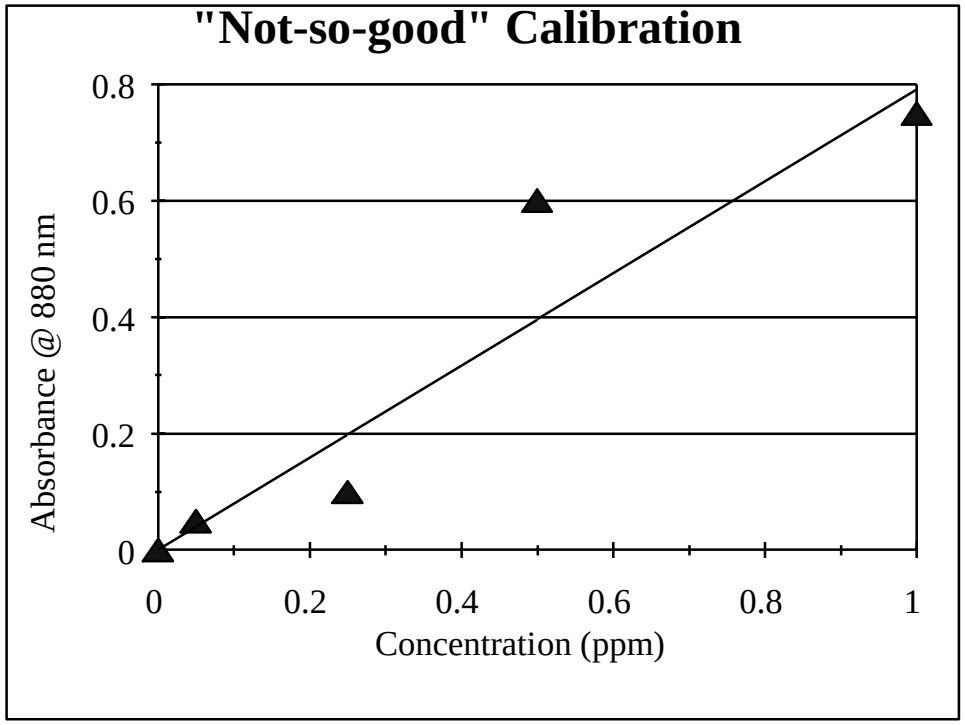
r= 0.98244

CALIBRATION - Evaluating the data

Simple Visual Evaluation

Data points should very closely fit the resultant calibration line.





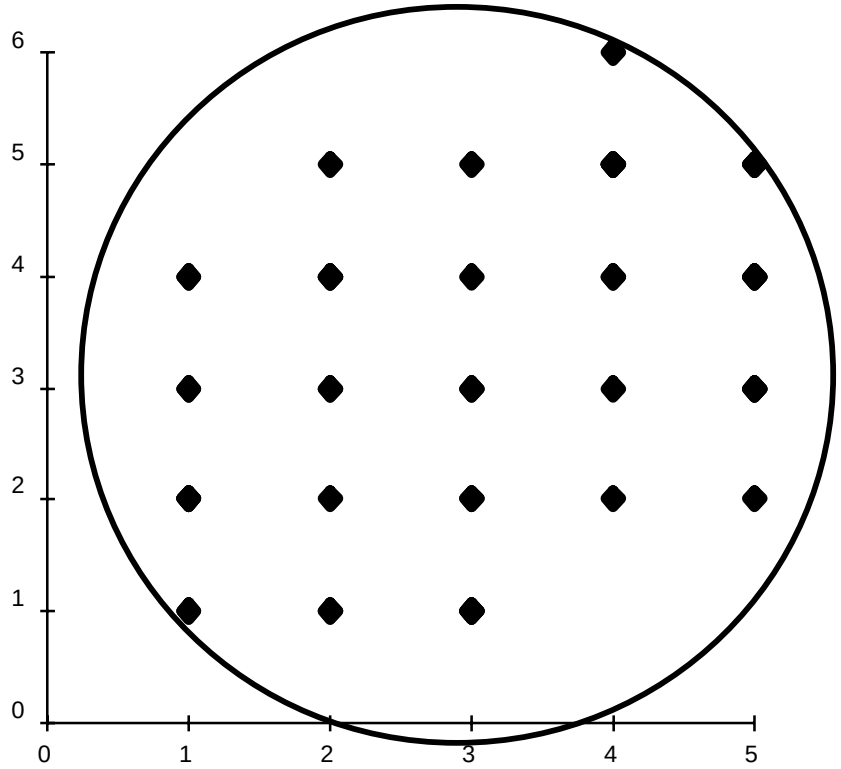
CALIBRATION - Evaluating the data

Statistical Evaluation

If using a linear regression, the correlation coefficient “r” provides a measure of the acceptability of a particular calibration curve.

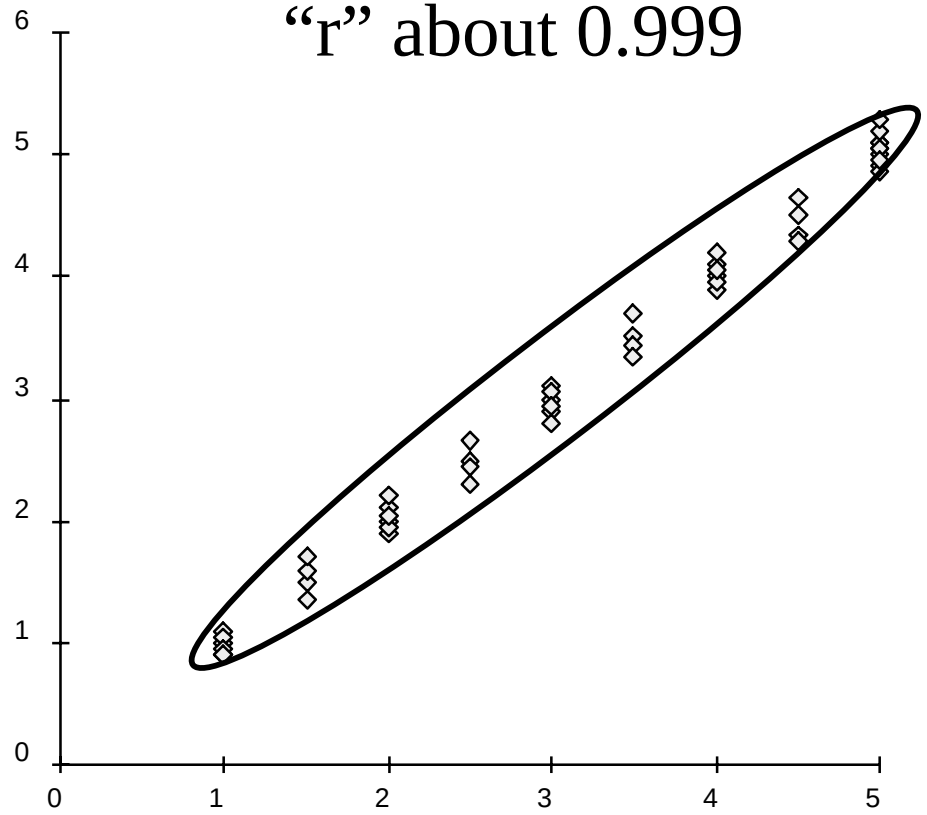
- “ r ” = complex mathematical equation
- Values between 0 (no correlation) and 1 (perfect correlation).
- Scientific calculators which offer 2-variable statistics can do it.
- A basic spreadsheet program function: Excel, Lotus, QuattroPro
- “r” must be 0.995 or greater

CALIBRATION - how the correlation coefficient works

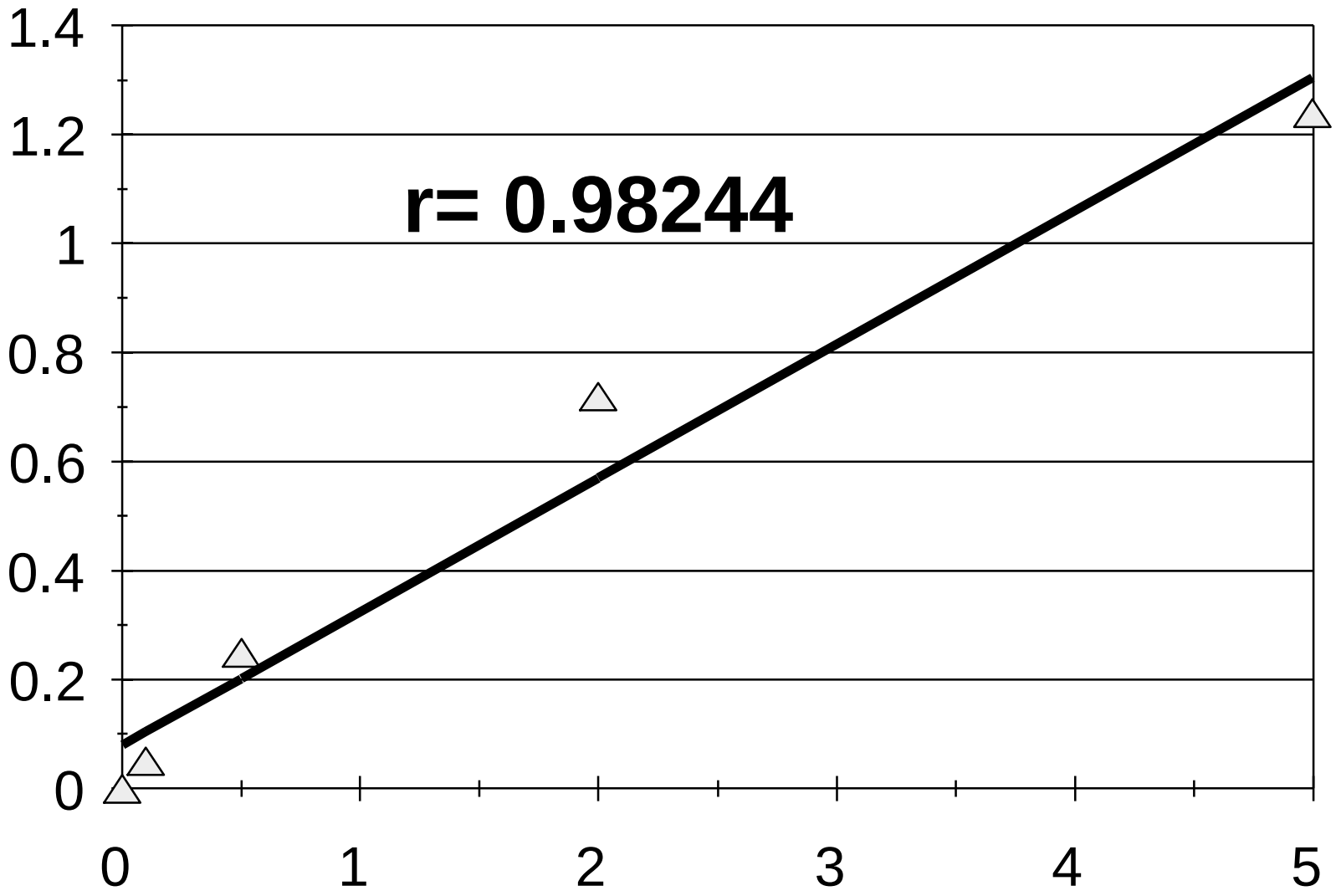


POOR correlation
"r" = 0

GOOD correlation
"r" about 0.999



CALIBRATION - Evaluating the data



Unacceptable correlation...will result in biased data

CALIBRATION - Evaluating the data

Analytical Evaluation - initial

- Analyze an independent check standard
- If regression, convert calibration standard responses to concentration

Converting calibration responses to concentration

Absorbance = 0.000:  2nd F (-0.328

Absorbance = 0.051:  2nd F (-0.119

Absorbance = 0.25:  2nd F (0.69

Absorbance = 0.72:  2nd F (2.61

Absorbance = 1.24:  2nd F (4.74

CALIBRATION - Evaluating the data

☆Compare recovery of the regression concentration relative to true value
☆Recoveries should generally be within 90-110%
☆It's more difficult to achieve ± 10% as you get close to the LOD

	True Concentration (mg/L)	Regression Concentration (mg/L)	Percent Recovery
Absorbance = 0.000:	0	-0.328	**???**
Absorbance = 0.051:	0.1	-0.119	**ND**
Absorbance = 0.25:	0.5	0.69	**138%**
Absorbance = 0.72:	2	2.61	**130.5%**
Absorbance = 1.24:	5	4.74	**94.8%**

CALIBRATION - Evaluating the data

Analytical Evaluation - ongoing

- Periodically confirm that response has not changed from initial
- Use a midpoint calibration standard (check standard) and blank
- Check at beginning, every 10 samples and end of each batch.

Recovery must be within 90-110% for phosphorus and ammonia.

$$\% \text{ Recovery} = \frac{\text{measured Value}}{\text{True value}} \times 100$$

CALIBRATION - Evaluating the data

What do I do if the check standard is outside the 90-110% range?

- Cease running samples until the problem is corrected
- Repeat initial calibration
- Repeat sample analysis since last acceptable calibration check

Additional Calibration Checks

- Analyze a reference sample (externally generated)
- Good time to run your blind standards

Detection Limits

Detection Limits

Limit of Detection (LOD) & Limit of Quantification (LOQ)

LOD

- ✓ Lowest concentration determined to be significantly different from a blank
- ✓ Formerly known as the MDL (Method Detection Limit)

LOQ

- ✓ Analyte concentration at which one can state with a stated degree of confidence that an analyte is present at a specific level in the sample tested.
- ✓ Defined in code [NR 149.03 (16)] as 10/3 times the LOD (i.e. 3.33 x LOD)

Detection Limits

Why is it important to know the LOD?

⇒ Confidence in results reported

⇒Alerts data-user to uncertainties or limitations of the data

⇒ Proper decisions can be made based on data

- Compliance decisions often made from pooled data
- What do you do with data of: **<25, 32, <40, 22.5, <50**

Detection Limits

Why is it important to know the MDL?

⇒ Censoring data biases data sets and restricts its usefulness

- Analyte X detected at 50 ppm.
- Decision is made to report < 100 ppm
  (which is certainly accurate)
- But what if the actual LOD is 10 ppm?

⇒ Potentially harmful levels of X may exist.....

but <u>below</u> our ability to detect them

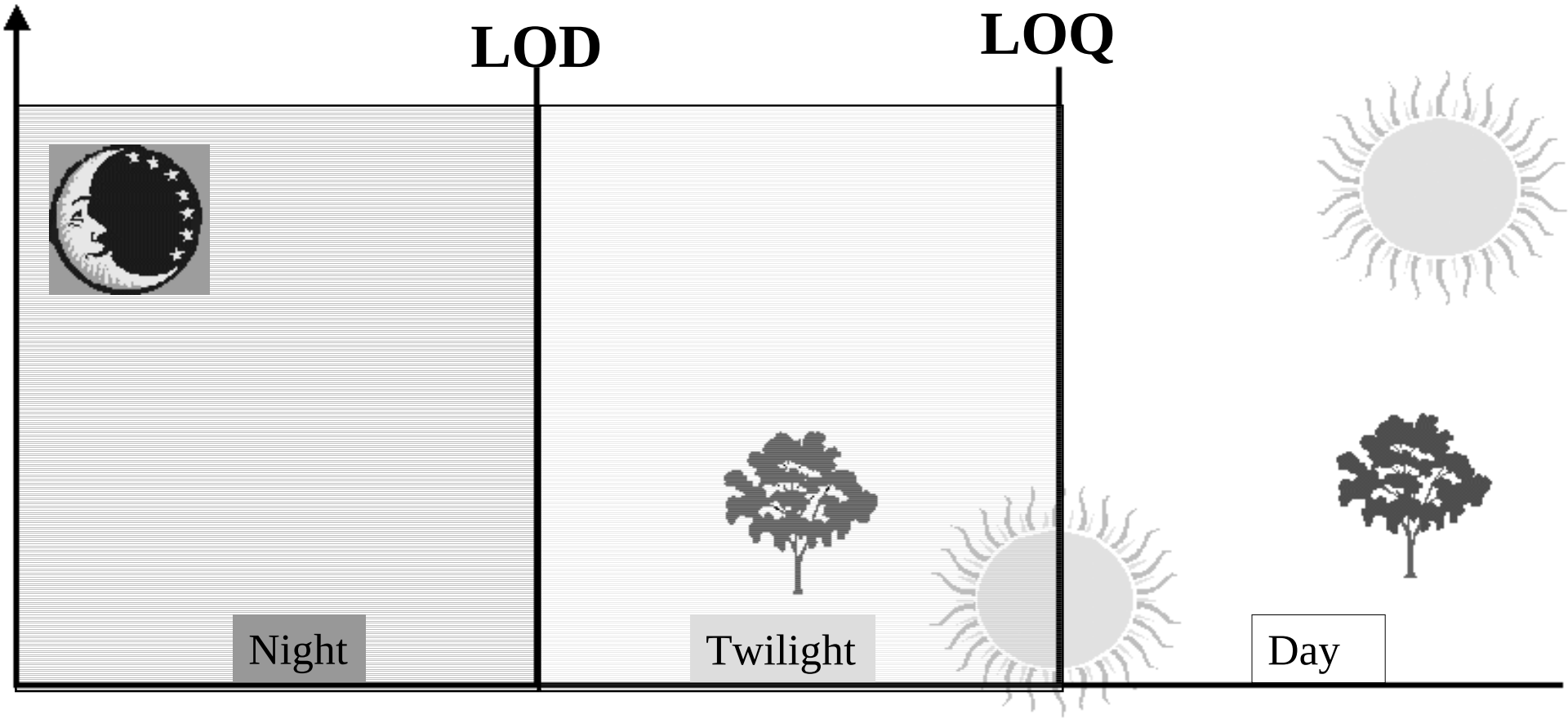
- To remediate this situation successfully, need to know.....
- <u>if</u> analyte is present
- <u>if</u> the concentration is *changing*

LOD/LOQ Interpretation

- **It isn't there**
  (NOT detectable)

- **It IS there**
- **Quantity is <u>uncertain</u>**
- **<u>Un</u>enforceable**
  (without multiple analyses)

- **It IS there**
- **Quantity IS certain**
- **Enforceable**



Detection Limits - Requirements to Determine & Report LOD

DNR
"The Department"

Lab Certification

NR149.11(5)

Labs MUST *determine* their LODs

NR149.15(3)

report down to the LOD...for a specified list of metals/organics

Assuming you have one dilution of 300 mL

Watershed Management

DMRs

BOD report to 2 ppm

NH3, TP: report to LOD
Default TP = 0.02 ppm
Default NH3= 0.10 ppm

Wastewater parameters: Specific examples

Procedure for BOD

Detection Limits - **BOD**

BOD detection limits are <u>theoretically based</u>.

- Assumption**:** the LEAST amount of depletion allowable is 2 mg/L.
- Based on the highest volume of sample used in a dilution series.
- This technique doesn't consider seed correction.

BOD bottle maximum volume!

$$\text{LOD mg/L} = 2 \text{ mg/L} \times \frac{300 \text{ mL}}{\text{mL sample}}$$

If the highest sample volume used is:	The LOD for **that** sample is:
300 mL	**2**
200	**3**
100	**6**
75	**8**
50	**12**

Detection Limits - **BOD**

Example 1:

Dilution 1 100 mL Depletion = 6.7 BOD = 20
Dilution 2 75 mL Depletion = 4.5 BOD = 18
Dilution 3 50 mL Depletion = 3.5 BOD = 21

What value should be reported?

What level of detection is there?

Assuming all three dilutions met depletion criteria,
report a BOD of 19.6 (20 + 18 + 21) / 3
LOD is 6 (based on: highest volume = 100 mL)

Detection Limits - **BOD**

Example 2:

Dilution 1	50 mL	Depletion:	< 2 mg/L
Dilution 2	25 mL	Depletion:	< 2 mg/L

What value should be reported?

What can be done in the future?

Report a BOD of " < 12"

Insufficient depletion; highest volume used is 50 mLs

Should be using more sample

Wastewater parameters: Specific examples

Procedure for TSS

Detection Limits - TSS

Like BOD, detection limits are <u>theoretically based</u>.

- Assumption**:** Minimum capture weight of 1 mg (of residue).
- Based on: Volume of sample filtered.

Thus, if 1 liter of sample is filtered, then you can "detect" 1 mg per liter.

$$\textbf{LOD mg/L} = 1000 \times \frac{\textbf{1 mg}}{\textbf{mL sample filtered}}$$

Volume filtered	Detection limit
25 mL	40 mg/L
50	20
100	10
250	4
500	2

Since most permit limits are about 30 mg/L, you will need to filter at least 50 mL of sample.

Wastewater parameters: Specific examples

Conventional procedure: TP, NH_3-electrode

Detection Limits - EPA procedure

1. Determine a spike concentration (close to the expected LOD)
2. Prepare at least 7 spiked replicates of reagent water at this spike level
3. Calculate the mean (**X**) and standard deviation (**SD**)
4. Obtain the "**t**"-value associated with the number of replicates
5. Calculate the LOD: **SD** times **t**
6. Perform the "5-point check" of the LOD

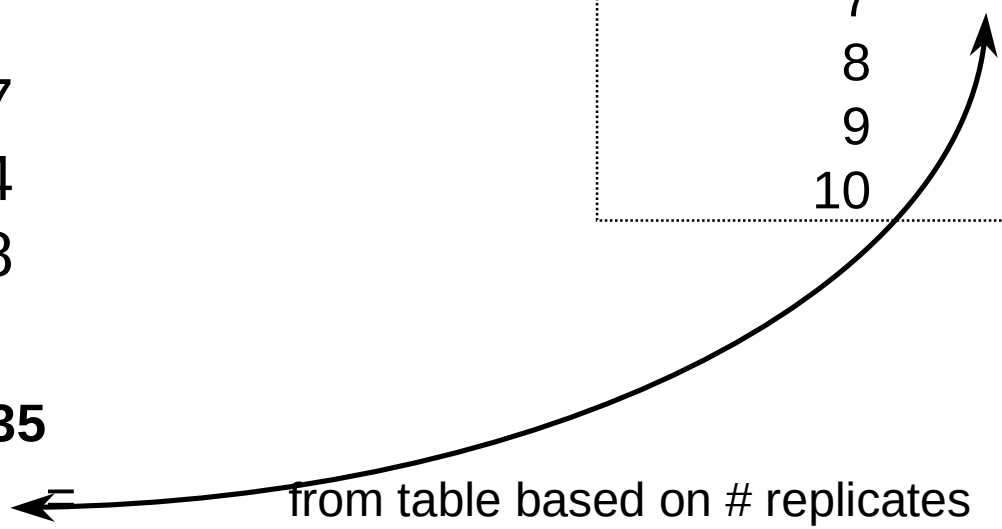
Detection Limits - EPA procedure

Ammonia Example

Spike level = 0.1 mg/L

Rep. 1	0.104
Rep. 2	0.082
Rep. 3	0.096
Rep. 4	0.1
Rep. 5	0.087
Rep. 6	0.114
Rep. 7	0.108
mean	**0.099**
st dev.	**0.01135**
t-value	**3.143**

# replicates	t-value
7	3.143
8	2.998
9	2.896
10	2.821



LOD= 0.035684 = t-value x std deviation

LOQ= 0.118948 = 3.333 x LOD

Discussion: If LOQ is theoretically equal to 10xSD, why doesn't that work here?

Detection Limits - Calculating Mean and SD on the Calculator

Going through the Ammonia LOD data

Set Mode to “1”: MODE [redacted]

Clear the registers: 2nd F DEL

Enter 1st value (0.104): [redacted] M+

Enter 2nd value (0.082): [redacted] M+

Enter 3rd value (0.096): [redacted] M+

Enter 4th value (0.100): [redacted] M+

Enter 5th value (0.087): [redacted] M+

Enter 6th value (0.114): [redacted] M+

Enter 7th value (0.108): [redacted] M+

Detection Limits - Calculating LODs with the Calculator

Obtain the LOD

✓ Get the Mean ($\bar{x}$).....: RCL 4 $\bar{x}$ = 0.098714285

✓ Get the standard deviation (SD): RCL 5 Sx= 0.011353623

✓ Calculate the MDL (SD x t)..: [redacted] **0.0356844**

Calculator window will say ANS * 3.143=

Now that you have the LOD, perform a 5-step procedure to determine whether or not the LOD is valid.

# replicates	t-value
7	3.143
8	2.998
9	2.896
10	2.821

Detection Limits - EPA procedure

The 5-point check (mandatory checks) **LOD= 0.036**

1. Is LOD greater than 10% of the spike level? **yes**

Spiked at **0.1**, so LOD should be > 0.01
If LOD < 10% of spike level, re-do at lower spike level

2. Is the spike level greater than the LOD? **yes**

Common sense: if LOD > spike level, couldn't detect it

3. Is the LOD below any relevant permit limit? **N/A**

(if there is one) Permit limit =_____

Detection Limits - EPA procedure

The 5-point check (additional checks)

Though not specifically required by the EPA method....
these checks help you obtain the best estimate of the LOD.

4. Is the signal-to-noise ratio (S/N) between 2.5 and 10? **yes**

S/N = Mean/std dev. S/N= 8.69

RCL 4 divided by **RCL 5 =** on your calculator

5. Is mean recovery within reasonably expected limits? **yes**

Mean recovery= mean/spike level x 100 = 98.71%

Expected range is approximately 80 to 120%

Accuracy (spikes)

Accuracy (Bias): Matrix Spikes - Discussion Points

⇒ Why do we do matrix spikes?

⇒ How much should I spike? (spike to background ratio)

⇒ How much can I dilute my sample?

⇒ Calculating recovery

⇒ What does my recovery mean?

⇒ Exercise : review calculation examples

DO NOT need matrix spikes for:

- BOD
- Suspended Solids

Accuracy (Bias): Matrix Spikes

Why: To evaluate the accuracy of method as influenced by specific matrices (sample types).

How: Add known amount of analyte to randomly selected routine samples

When: 5% of samples (1 per 20 samples)

NOTE: Raw + effluent = 2 samples

What:

1. Calculate % recovery
2. Evaluate performance against control limits

Accuracy (Bias): Matrix Spikes

How <u>much</u> should you spike?

1. Consider the upper calibration range!

If you calibrate to 1 ppm...
your effluent is about 0.25 ppm....
and you spike at 1.0 ppm.....you will exceed the calibration range.

2. Spike at 1 to 5 times the level in the sample

If your effluent averages 1 ppm, spike between 1 and 5 ppm

3. Limit additional volume to <10% of sample

(i.e., 1 ml standard + 10 ml sample)

- If you spike at less than 1x, risk the ability to recover it.
- As spike increases beyond 5X, TOO easy to recover.

Accuracy (Bias): Matrix Spikes

Background correction

- If you dilute spike (with sample) to a known volume....
  sample concentration must be adjusted.

- If the spike is added "on top of" the sample
  (i.e., 100 mL + 10 mL)....
  the *spike concentration* must be adjusted as well.

- If dilution from the spike is kept to 1% or less...
  direct subtraction of unspiked sample is allowed.

Accuracy (Bias): Matrix Spikes

The goal of a matrix spike is to provide us with information regarding how accurate our sample analysis results are.

If spike recovery is only 50%, then the potential exists that the true concentration of the target parameter is as much as twice the measured concentration.

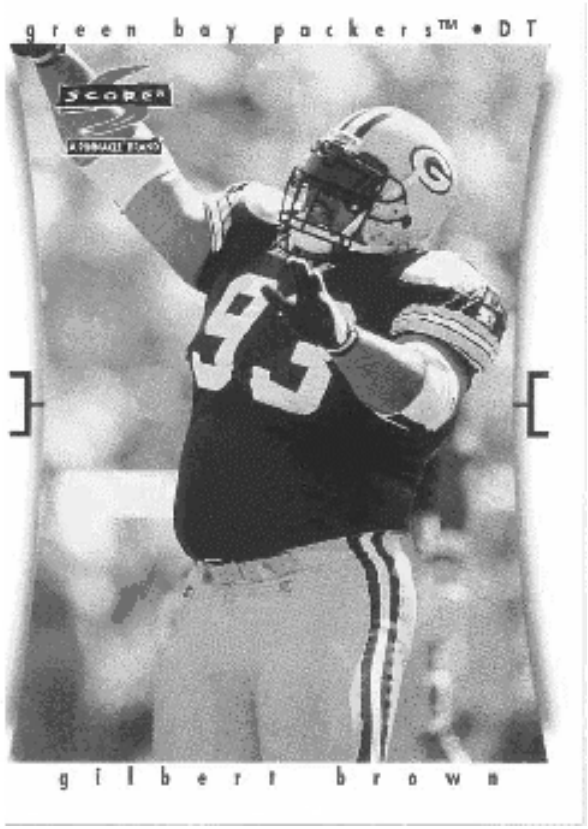
Dilution - How much is too much?

- ✔ The bottom line is that you want to use as much sample for the matrix spike as you did for the sample itself.
- ✔ References vary on how much dilution is TOO much.
- ✔ Our guidance has been to limit dilution to 10% or LESS.

We frequently see laboratories that prepare spikes by diluting a sample 50:50 with the spike solution. The next slide provides a more graphic explanation for why this is inappropriate.

Matrix Spike II: The solution is NOT dilution

starring:



“100% Matrix”

Gilbert Brown

Packers DT

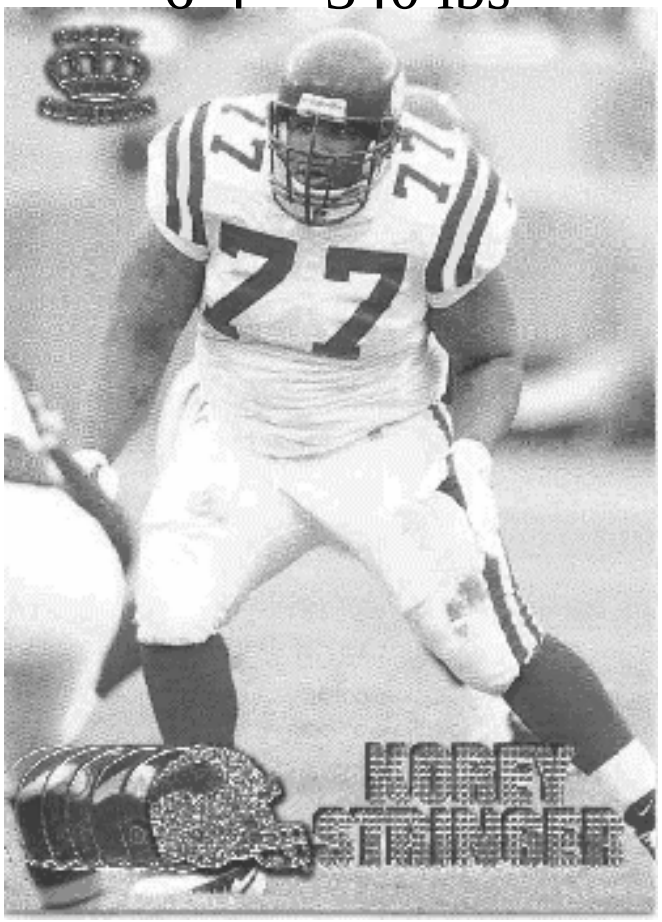
6’2” 325 lbs

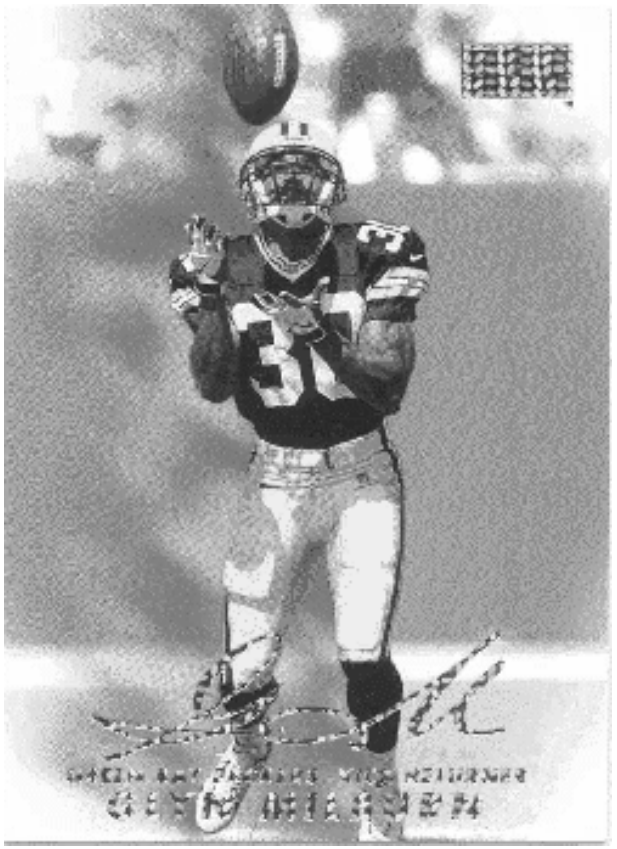
“Matrix Spike”

Korey Stringer

Vikings (boo!) OT

6’4” 340 lbs





“50% Diluted Matrix”

Glyn Milburn

ex-Packer KR

5’8” 177 lbs

Accuracy (Bias): Matrix Spikes

Calculation of % Recovery

$$\% \text{ Recovery} = \frac{\text{Spiked Sample} - \text{Unspiked sample}}{\text{Amount of spike added}} \times 100$$

seems simple enough.....BUT.....

Results will vary depending on whether you

- simply add the spike on top of the sample, or you
- add the spike first, then dilute to a fixed volume with sample, and
- is further compounded by whether or not you are digesting

Remember: Do NOT perform any rounding until the last step!

In-class example

Analysis:	NH_3-electrode
Unspiked sample result:	2.0 mg/L
Volume of sample:	50 mL
Concentration of spike solution:	25 mg/L
Volume of spike solution added:	5 mL
Total Volume of sample + spike:	55 mL
Spiked sample result:	4.25 mg/L

$$= \frac{4.25 - (2.0 \times 0.90)}{(25 \times 0.0909)} \times 100 = 107.0\%$$

or: $\frac{233.75 - 100}{125} \times 100 = 107.0\%$

If you got 90%, you didn't account for dilution of EITHER the sample or the spike
If you got 98% you accounted for dilution of the sample but not for the spike
If you got 99%, you accounted for dilution of the spike, but not for the sample

Accuracy (Bias): Matrix Spikes - Calculations

A. Determine the # of ug (of analyte) **in the unspiked sample**

Multiply the unspiked sample concentration by the mLs of sample used

B. Determine the contribution (ug) from the sample in the spike

1. Subtract the mLs of the spike from the total mLs of sample + spike

2. Multiply the answer from #1 above by the sample concentration

C. Determine the # of ug (of analyte) **spiked**

Multiply the concentration of the solution used to spike by the # mLs spiked

D. Determine the # of ug (of analyte) **in the spiked sample**

Multiply the spiked sample concentration by the # mLs of this sample

E. Determine the # of ug (of analyte) **recovered**

Subtract "B" from "D"

% Recovery = *Divide "E" by "C" and multiply by 100*

key relationship: mg/L = ppm =ug/mL

Matrix Spikes: Ammonia example- diluting to known volume

Unspiked sample **3.263 ug/mL** **Spiked sample** **5.625 ug/mL**
Unspiked Sample Volume **100 mL** **Spiked sample volume** **100 mL**

Spike volume **20 mL**
Spike Conc. **20 ug/mL**

A. Determine the # of ug (of analyte) **in the unspiked sample = 326.3**
3.263 ug/mL X 100 mL

B. Contribution (ug) from the sample in the spike = 261.0
3.263 ug/mL X (100 mL - 20 mL) = 3.263 X 80

C. The # of ug (of analyte) **spiked = 400**
20 ug/mL X 20 mL

D. The # of ug (of analyte) **in the spiked sample = 562.5**
5.625 ug/mL X 100 mL

E. The # of ug (of analyte) **recovered = 301.5**
562.5 - 261.0

% Recovery = 75.375%
(301.5 / 400) X 100

Matrix Spikes: Ammonia example- adding "on top"

Unspiked sample 2.0 ug/mL **Spiked sample 4.25 ug/mL**
Unspiked Sample Volume 50 mL **Total volume 55 mL**

Spike volume 5 mL
Spike Conc. 25 ug/mL

A. Determine the # of ug (of analyte) **in the unspiked sample = 100**
2.0 ug/mL X 50 mL

B. Contribution (ug) from the sample in the spike = 100
2.0 ug/mL X (55 mL - 5 mL) = 2.0 X 50

C. The # of ug (of analyte) **<u>spiked</u> = 125**
25 ug/mL X 5 mL

D. The # of ug (of analyte) **in the spiked sample = 233.75**
4.25 ug/mL X 55 mL

E. The # of ug (of analyte) **<u>recovered</u> = 133.75**
= D - B = *233.75 - 100*

% Recovery = 107.0%
= E / C =*(133.75 / 125) X 100*

Matrix Spikes: Ammonia example- adding "on top" the conventional calculation

Unspiked sample 2.0 ug/mL **Spiked sample 4.25 ug/mL**
Unspiked Sample Volume 50 mL **Total volume 55 mL**

Spike volume 5 mL
Spike Conc. 25 ug/mL

A. Correct the concentration in the unspiked sample = 1.81818
2.0 ug/mL X (50/55) mL = 2.0 x 0.90909

B. Correct the spike concentration = 2.272727
25 ug/mL X (5/55) mL = .090909

C. Calculate recovered concentration = 2.43182
4.25 ug/mL - 1.81818 ug/mL

% Recovery = 107.0%
= (C - B) x 100 =*(2.43182 / 2.272727) X 100*

Matrix Spikes: Phosphorus example

Unspiked sample .246 X 25 = 6.15ug/mL **Spiked sample .346x 25= 8.65 ug/mL**
Unspiked sample Volume 2mL=>50 mL **Total volume 2 mL + 1 mL =>50 mL**

Spike volume 1 mL
Spike Conc. 5 ug/mL

A. Determine the # of ug (of analyte) **in the unspiked sample = 12.3**
0.246 ug/mL X 50 mL

B. Contribution (ug) from the sample in the spike = 12.3
0.246 ug/mL X 50 mL

C. The # of ug (of analyte) **spiked** = **5**
5 ug/mL X 1 mL

D. The # of ug (of analyte) **in the spiked sample = 17.3**
0.346 ug/mL X 50 mL

E. The # of ug (of analyte) **recovered** = **5**
= D - B = *17.3 - 12.3*

% Recovery = 100.0%
= E / C =*(5 / 5) X 100*

Precision (Replicates)

Precision: Replicates

Why: Used to evaluate repeatability (reproducibility)

How: Analyze randomly selected routine samples in duplicate (including digestion steps)

When: 5% of samples (1 per 20 samples)

NOTE: Raw + effluent = 2 samples

What: 1. Calculate Range (or RPD)
2. Evaluate performance against control limits

NOTES:
1. Replicates are frequently termed “Duplicates”. The terms are interchangeable.
2. Precision is concentration dependent

Precision: Replicates

Evaluating Replicates

Based on absolute difference (Range) or Relative percent difference (RPD) between duplicates

Example

Sample = 22

Replicate =18

Range

expressed in same units as values

= Absolute Difference

= Larger value – smaller value

Range = 22 - 18 = 4

RPD

expressed as %

$$RPD = \frac{\text{Range}}{\text{Mean of the replicates}} \times 100$$

RPD = Range / Mean

Range = 4

Mean = (22 + 18)/2

= 20

RPD = (4/20) x 100

= 20%

Precision: Replicates

"Precision is concentration dependent"

Which is a brief way of saying that precision is difficult to evaluate without knowing the concentration levels involved.

Consider the analysis of TSS

- The range of replicates is 200 mg/L
- First thought: "Gee...that's terrible!"
- But.....what if the two values were 12,400 and 12,600?
- Now 200 doesn't look so bad.
- But....your opinion changes if the two values are 250 and 50

Precision: Replicates

Dealing with concentration dependency

- Separate control limits based on concentration
  Ex. effluent phosphorus is about 0.05; influent about 5 ppm
- Best place to start is at 10 x the LOD or LOQ
- MAY need <u>more</u> than two levels

  But you don't want so many different levels that you will never generate enough data to create your own limits either

One laboratory has 7 sites requiring BOD

- ✓ 1 site 10-50 BOD
- ✓ 4 sites range 30-200 BOD
- ✓ 1 site 350-700 BOD
- ✓ 1 site 900-3000 BOD

this situation may need 3 or even 4 concentration level ranges

Control Limits

Do I need control charts?

NO..... but.....

- ☺ Control chart use is "strongly encouraged"
- ☺ Control charts are useful in heading off problems
- ☺ Control charts provide a visual tool

Control Limits

What exactly DO I need? NR 149.14 (3) (g)

Quality control limits
for replicate sample and spiked sample analysis
shall be calculated for each matrix type
using a method from an authoritative source
[NR 149.03 (5) (a - w)].

.....

When quality control data
shows a dependency on concentration,
the laboratory **shall** calculate separate control limits
to address the concentration dependency.

.....

Control Limits

What exactly DO I need? NR 149.14 (3) (g)

For labs with

- less than 20 quality control results
- within 12 months,

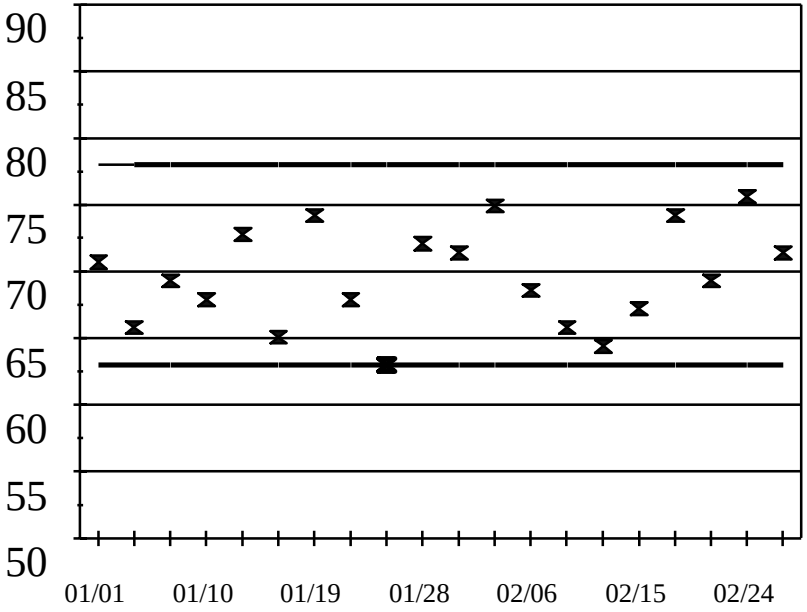
the laboratory **may** set quality control limits based on

- information given in the authoritative sources,
- laboratory experience, or
- the experience of other laboratories.

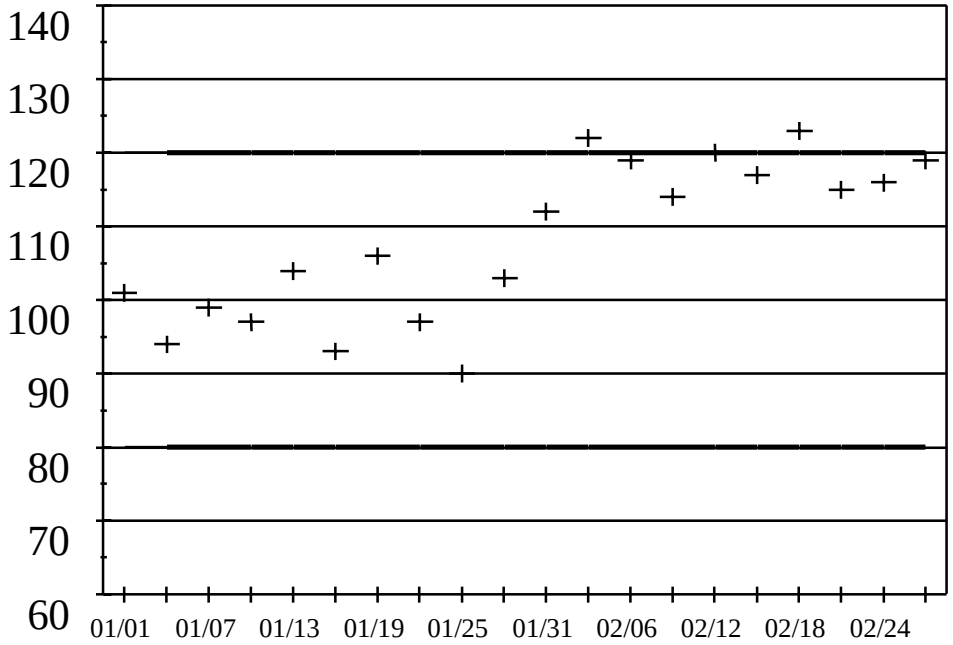
Some sample control charts.....

Evaluation of control charts can often be accomplished by a simple quick visual inspection

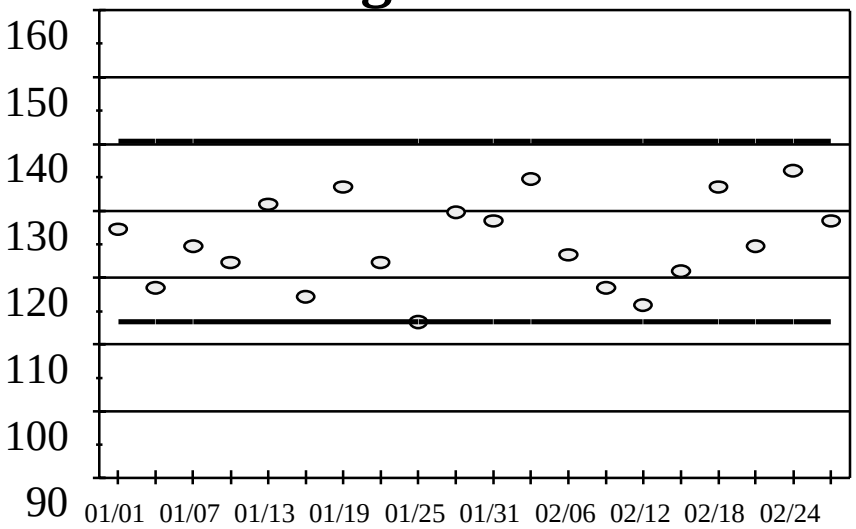
Low Bias



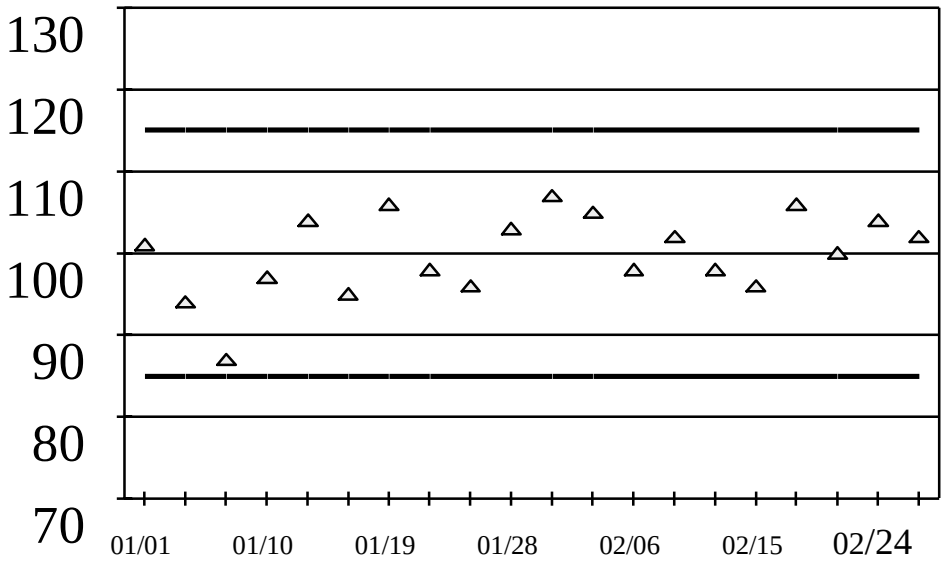
Significant change



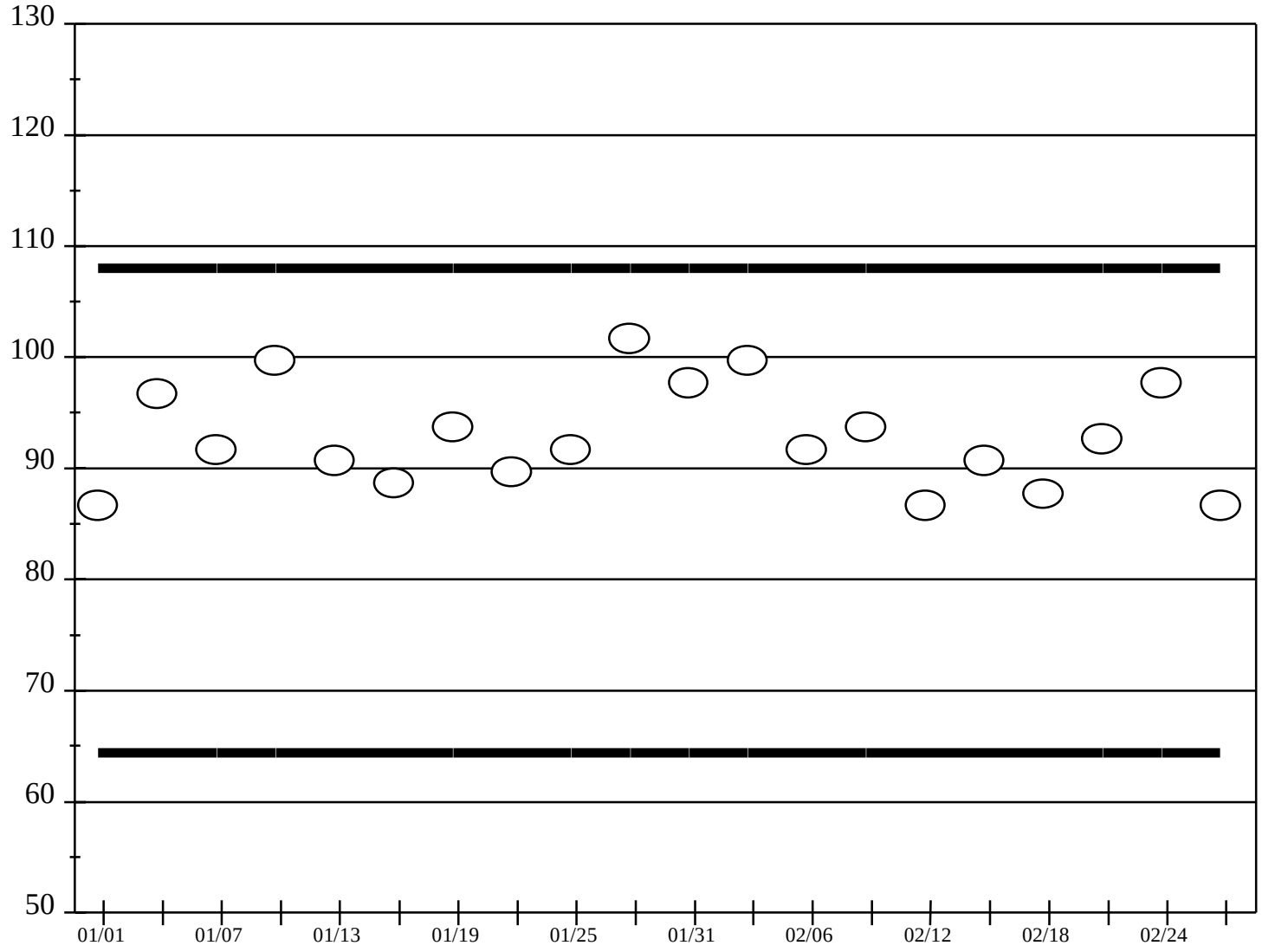
High Bias



Slight trend



Is this analysis “in control”?



N = 20 SD = 7.28 Mean = 88.85 Limits = 67 to 110.7%

How are control limits calculated?

Matrix spike & RPD Control limits

1. Test the data for and eliminate outliers before proceeding.
2. Calculate the mean and standard deviation of the data.
3. Warning limits = Mean $\pm$ 2 standard deviations
4. Control limits = Mean $\pm$ 3 standard deviations

NOTE: RPD is a 1-tailed test, so only Mean +

Range Control limits

1. Test the data for and eliminate outliers before proceeding.
2. Calculate the mean of the data.
3. Warning limits = 2.51 x Mean
4. Control limits = 3.27 x Mean

What if I don't have enough data?

If you do QC any less frequently than once every 2 weeks, you will not have enough data.

Use limits from an authoritative source

Standard Methods 18th ed., Table 1020I

- Spikes: 80-120%
- RPD (high concentration): ± 10%
- RPD (low concentration): ± 25%

Use limits from an adjacent facility

Only do this if the facility has similar processes to your own and they are using the same procedure as you are.

Set limits based on your own lab experience

Be prepared to defend what you've come up with!

Control Limits

A lab auditor's dream: What if limits are TOO tight?

1. Be sure that you are not excluding <u>out-of-control</u> data!

   Outliers should be excluded, but

   all out-of-control points are **not** outliers.

2. Include enough significant figures.
   - If values are whole numbers (e.g., 10, 89%), you can use one or even two decimal points to include some variability (10**.2**, 89**.3**%).
   - This same problem has been observed when a laboratory only reported recoveries to the nearest 5%.

Control Limits - Spike Limits example

Exercise:

Calculate matrix spike control limits from the following set of data

Mean	=	98.05	
std dev (SD)	=	5.1245025	
2 x SD	=	10.249005	
3 x SD	=	15.3735075	
Mean - 3SD	=	98.05 - 15.374	= 82.676
Mean + 3SD	=	98.05 + 15.374	= 113.424

Control Limits should be 82.7 *to* 113.4

Warning Limits should be 87.8 *to* 108.3

Matrix spikes

Data #	%R
1	**92**
2	**94**
3	**101**
4	**99**
5	**107**
6	**102**
7	**96**
8	**99**
9	**91**
10	**105**
11	**103**
12	**97**
13	**100**
14	**89**
15	**94**
16	**98**
17	**90**
18	**101**
19	**104**
20	**99**

Control Limits - Replicates

$$\text{RPD} = \frac{\text{Range}}{\text{Mean of the replicates}} \times 100$$

	Sample	Replicate	Range	RPD
1	12	11	**1**	8.70%
2	10	8	**2**	22.22%
3	14	12	**2**	15.38%
4	10	9	**1**	10.53%
5	10	11	**1**	9.52%
6	9	8	**1**	11.76%
7	11	8	**3**	31.58%
8	14	11	**3**	24.00%
9	13	13	**0**	0.00%
10	8	9	**1**	11.76%
11	10	12	**2**	18.18%
12	15	13	**2**	14.29%
13	11	12	**1**	8.70%
14	10	12	**2**	18.18%
15	8	8	**0**	0.00%
16	10	11	**1**	9.52%
17	9	11	**2**	20.00%
18	10	11	**1**	9.52%
19	12	15	**3**	22.22%
20	11	11	**0**	0.00%

Exercise:
Calculate replicate control limits from the following set of data

Range Control Limits

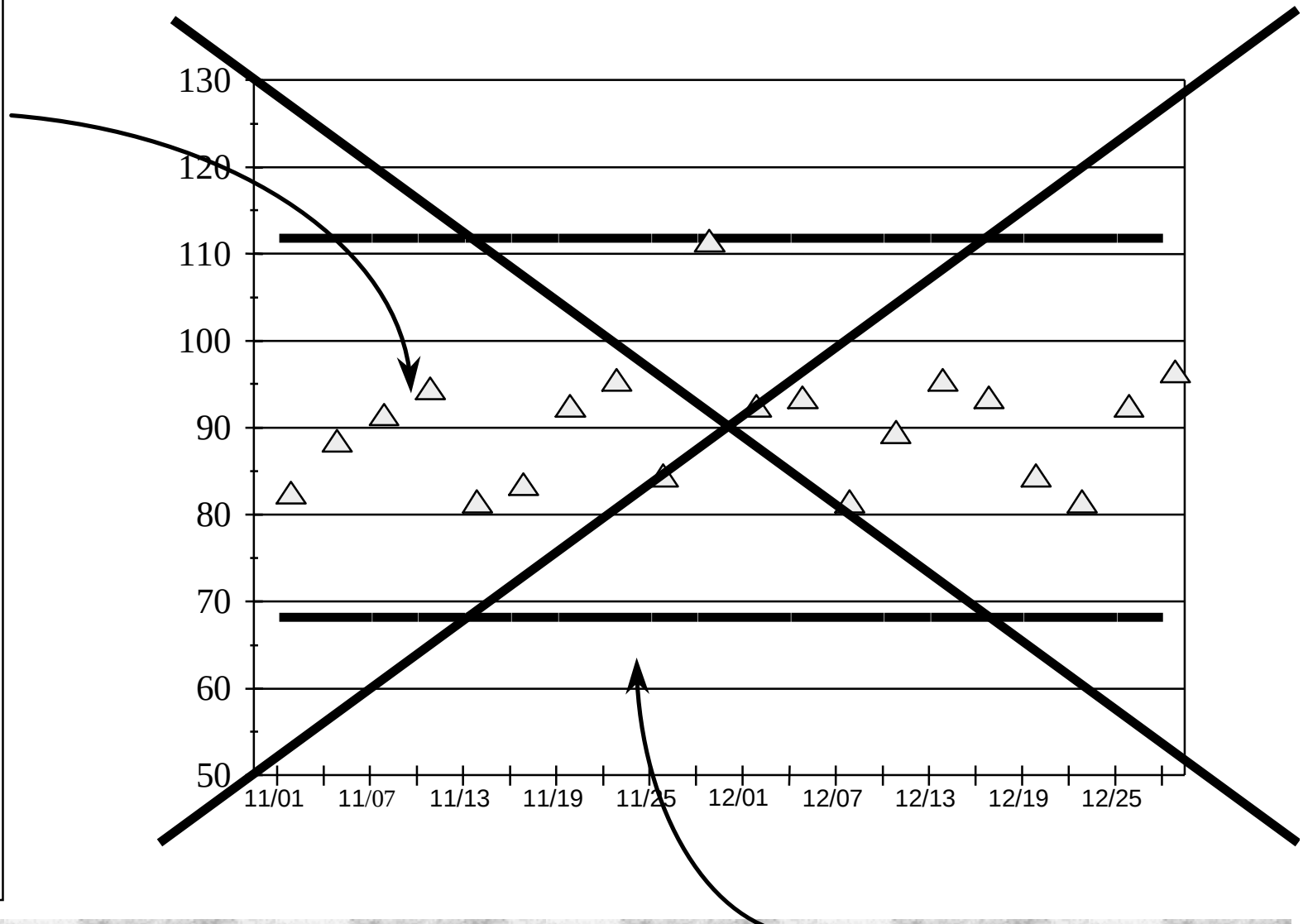
Mean	1.45	
Warning	3.64	**1.45 x 2.51**
Control	4.74	**1.45 x 3.27**

RPD Control Limits

Mean	13.3%	
Std dev.	8.4%	
2SD= 16.8, 3SD=25.2		
Warning	30.1%	**13.3+16.8**
Control	38.4%	**13.3+25.2**

DO NOT plot the data used to create limits against those same limits!

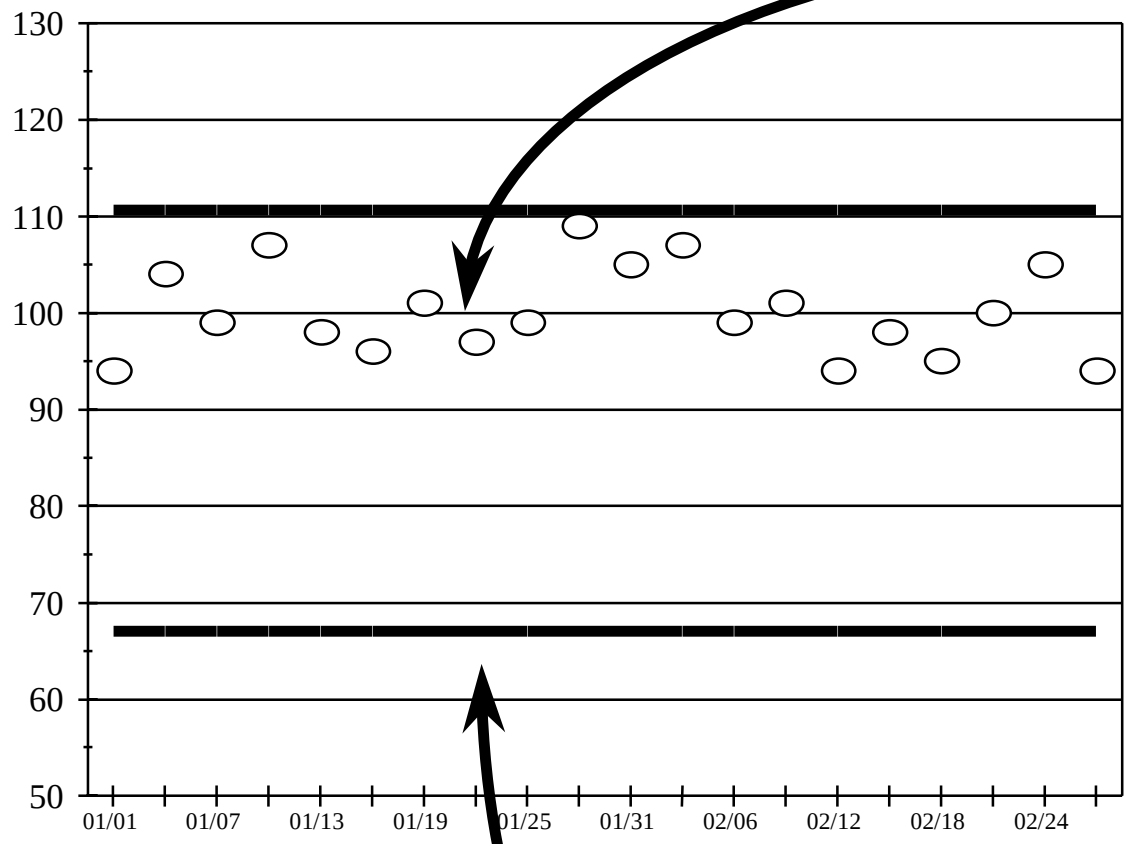
DATE	%R
11/01	81
11/04	87
11/07	90
11/10	93
11/13	80
11/16	82
11/19	91
11/22	94
11/25	83
11/28	110
12/01	91
12/04	92
12/07	80
12/10	88
12/13	94
12/16	92
12/19	83
12/22	80
12/25	91
12/28	95



N = 20 **SD = 7.28** **Mean = 88.85** **Limits = 67 to 110.7%**

DO plot data from period B-C against the limits created from data set A-B!

DATE	%R
11/01	81
11/04	87
11/07	90
11/10	93
11/13	80
11/16	82
11/19	91
11/22	94
11/25	83
11/28	110
12/01	91
12/04	92
12/07	80
12/10	88
12/13	94
12/16	92
12/19	83
12/22	80
12/25	91
12/28	95



DATE	% R
01/01	94
01/04	104
01/07	99
01/10	107
01/13	98
01/16	96
01/19	101
01/22	97
01/25	99
01/28	109
01/31	105
02/03	107
02/06	99
02/09	101
02/12	94
02/15	98
02/18	95
02/21	100
02/24	105
02/27	94

N = 20 SD = 7.28 Mean = 88.85 Limits = 67 to 110.7%

Control Limits - Dealing with outliers

There are many statistical tests available for identifying outliers. One that is relatively easy to use is the Grubbs test.

$$Z = \left| \frac{\text{mean - questionable data point}}{SD} \right|$$

- Only test the highest (and/or lowest) value
- Ignore the sign of the "Z" value….is always "+"
- Include the suspected outlier when calculating mean and SD
- If the calculated Z-value is greater than the criterion Z value for that number of data points, then the value is an outlier

N	Critical Z
18	2.65
19	2.68
20	2.71
21	2.73
22	2.76
23	2.78
24	2.80
25	2.82
26	2.84
27	2.86
28	2.88
29	2.89
30	2.91
35	2.98
40	3.04
50	3.13
60	3.20

Control Limits - Dealing with outliers

Exercise

DATE	%R
11/01	81
11/04	87
11/07	90
11/10	93
11/13	80
11/16	82
11/19	91
11/22	94
11/25	83
11/28	110
12/01	91
12/04	92
12/07	80
12/10	88
12/13	94
12/16	92
12/19	83
12/22	80
12/25	91
12/28	95

1. Calculate the mean and SD

Mean = 88.85 SD = 7.278 Limits =67 - 110.7

2. Test the high value (110)

$$Z = \frac{110 - 88.85}{7.278} = 2.9058$$

N	Critical Z
18	2.65
19	2.68
20	2.71

3. Test the low value (80)

$$Z = \frac{88.85 - 80}{7.278} = 1.2159$$

4. Discard outliers; re-calculate mean and SD

Since Z_{110} > criterion, 110 is an outlier

Mean =87.737 SD= 5.4553 Limits = 71 - 104

NOTE: Step 4 may also require a re-check for additional outliers!

Corrective Action

Corrective Action

What IS Corrective Action?

- ✓ In a nutshell, Corrective Action is anything done in response to an out-of-control situation.
- ✓ It MUST, however, be designed to identify the reason for the failure, and then correct it.
- ✓ There should also be a plan to quickly verify that the action taken has the desired effect.

What Corrective Action is NOT

- ☞ Simply labeling all matrix spike failures as “matrix effects”
- ☞ Checking calculations only
- ☞ Simply re-running the samples

Corrective Action

What do I do with the data?

NR 149.14(3) (h, i) requires the laboratory to:

- Repeat all samples
  - back to the last valid QC sample **of the same type**
  - unless you can show that ONLY that one sample is affected
- If samples cannot be re-analyzed....
  - results must be qualified back to the acceptable check.

DMRs require you to...

- mark the “QC Exceedance” box, and also
- identify (*) all sample results that are affected.

What else can I do?

☞ **You may wish to**

...increase the frequency of QC sample analysis

...above the minimum

...to minimize the amount of data which must be qualified

...in the event of an exceedance.

☞It's a good idea to create some sort of logbook or form to document these situations.

- HOW did you become aware of the problem?
- WHAT action did you take to fix the problem?
- HOW do you know the problem has been resolved?

Corrective Action

Situation	**Corrective Action**
BOD: GGA failing high	1) Was initial calibration done properly? 2) Change in seed source? 3) Possibility of nitrification? 4) Qualify data on DMR back to last good GGA.
NH_3 electrode slope < -54 mV	1) Check that membrane is intact; no bubbles. 2) Make sure fresh filling solution is used. 3) Is the electrode stablizing normally? too slow? 4) Is the intercept climbing above the LOD?
Phosphorus calibration.... "r" is <<<0.995	1) View plot...does a single standard look funny? 2) Beyond linear range? (about 1 ppm for most) 3) Contamination..especially at low level?

Documentation

Documentation

A laboratory is required to:

maintain records: **NR 149.06 [esp. (5)]**

- which are un-alterable,
- which enable complete traceability [by an auditor]
- for a given three-year compliance period

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Operating Principles

- If you didn't document it, you didn't do it
- You did the work.....take credit for it!

Documentation

But.... why? No one ever looks at this stuff....

- Consider it insurance: pay it and hope you won't need it
- If your data comes under scrutiny, you lose without it
- It can actually help identify problems
- Your auditor WILL look at this
- Provides credibility for test results
- Shows you are doing the test properly
- *Keeps those DNR auditors off your back!*

Documentation

The obvious (basic items to document**)**

- Refrigerator temperature
- Oven temperatures
- Balance calibration verification
- Raw data from sample analysis
- When the membrane is changed on the DO probe

Documentation

The not-so-obvious

Corrective actions taken

if an auditor asks what you did in response to a GGA failure...
can you ***show*** them? (ability to *tell* doesn't count!)

Historical QC limits

can you find control limits in use 3 years ago?

Blind Sample Performance History

Can you show an auditor your performance over the past 3 years?

Instrument maintenance

Documentation

Spike preparation - often overlooked

What the code says: [NR 149.06 (1)(intro.)]

Records to be retained include but are not limited to records of the following:

(b) Quality control data for spikes, replicates, method blanks, blind standards, reference samples, calibration standards and known standards. Quality control results shall be traceable to all of the associated sample results.

What it means (as it relates to spikes):

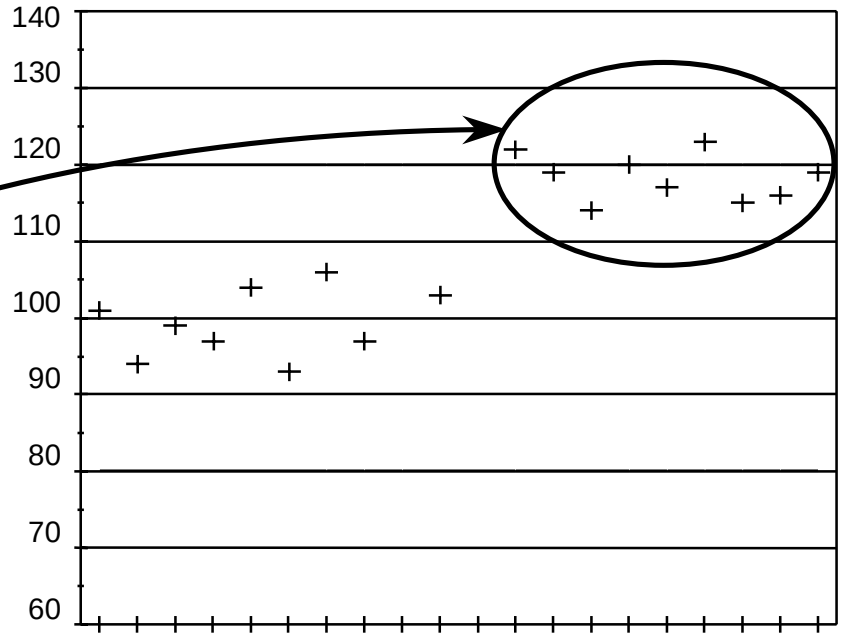
An auditor must be able to verify spike concentration, which means

- Concentration of the solution used to prepare spikes
- Information necessary to show that spike solution had not expired.
- The volume of spike solution used
- The volume of sample used
- The final volume of sample + spike
- The sample that was used to prepare the spike

Documentation

Documentation Exercise

What happened here?



Possible Causes

How would you know (Documentation)

Summary

☑ Formulate a gameplan [your QA manual]
☑ Calibrate (and evaluate it)
☑ Determine your detection capability
☑ Determine your accuracy
☑ Determine your precision
☑ Evaluate your precision and accuracy
☑ Implement a corrective action plan
☑ Provide documentation

Questions?